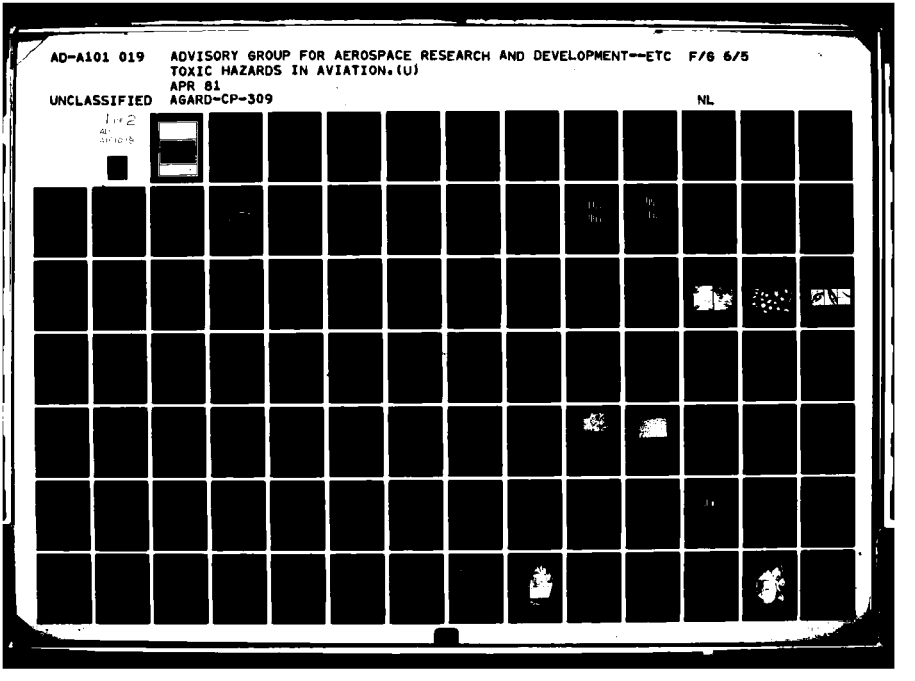
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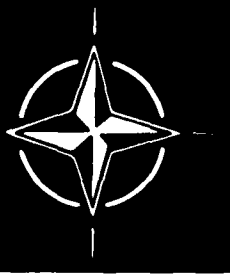

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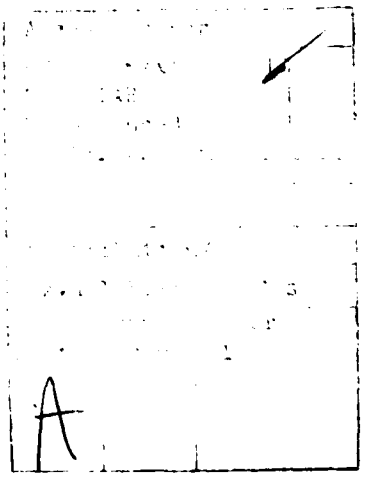

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PREFACE

The aviation environment has always contained many toxic materials and products. With the evolution of more advanced aircraft propulsion mechanisms, specialised aircraft material development and associated maintenance activities, there has been a major increase in the potential toxic hazards associated with these systems. The threat of toxic exposure covers the entire spectrum of low-level continuous or intermittent to high-level brief accidental or unavoidable exposures.

However, the protection of the crew and passengers is not the only concern in dealing with the toxic hazards in aviation. Responsibilities include research to address the biomedical aspects of occupational health and safety standards, toxic substances, environmental impact criteria and classification of transportation.

Papers presented at the Aerospace Medical Panel's Specialists' Meeting held in Toronto, Canada, 15–19 September 1980.

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RAMJET FUEL TOXICOLOGY

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SUMMARY

New aircraft and ramjet fuels are being developed which contain high density components. These fuels contain isomers of perhydromethylcyclopentadiene (RJ4), reduced dimers of bicyclohepadiene (RJ5), a tricyclodecane compound (JP10) and methylcyclohexane. Methylcyclohexane is by far the most volatile of the constituents studied having from 100 to 1500 times more vapor pressure than the other components. This fact is paramount to considerations of relative hazards.

Each of the compounds have been studied for their acute, subacute, and chronic toxicity. The agents are of low order toxicity from acute exposure. Chronic toxicity studies were run on RJ4, RJ5, and JP10 using rats, mice, dogs and monkeys.

The results of these studies demonstrate the low order of toxicity of these constituents. Kidney and liver hyperplasia in RJ4 exposed rats and pulmonary irritation in dogs and monkeys exposed to RJ4 and RJ5 emerge as the salient results of these studies. Although there is some indication of increased tumor incidence in a small number of mice held for one year after exposure to near saturated RJ5 vapors, there is no clear cut evidence that this compound is carcinogenic. The finding of respiratory irritation should be considered relative to possible human experience of chronic exposure to RJ4 or RJ5. Due to their low vapor pressures, the inhalation hazard (the probability of injury in use) is extremely low. The odors of RJ4, RJ5, and JP10 are extremely objectionable and it is doubtful that workers would tolerate concentrations far less than those used in these studies. These fuels show a relatively low order of toxicity in experimental animals and are judged to be of low inhalation hazard to man.

Our Laboratory and the US Navy Toxicology Unit of the Naval Medical Research Institute have been performing work on the toxicology of various constituents of ramjet fuels as well as conventional jet fuels since the late 1960s. This paper will attempt to present the data necessary to formulate the relative hazards of these compounds and relate those hazards to those found with the well known US Air Force aircraft jet fuel, JP4.

The formulation for JP9 (ramjet) fuel was originally a mixture of 70% RJ4, 20% RJ5 and 10% methylcyclohexane. Later RJ4 was replaced by JP10 in the formulation. RJ4 (TH dimer) contains isomers of perhydromethylcyclopentadiene. RJ5 is a mixture of reduced dimers of bicycloheptadiene and is also known as "Shelldyne H." JP10 is a tricyclodecane compound. The physical properties are found in Table 1.

Methylcyclohexane (MCH) is by far the most volatile of the constituents studied having from 100 to 1500 times more vapor pressure than the other components. This fact is paramount to considerations of relative hazards. MCH has low oral toxicity in the rabbit with the lowest lethal dose published at 4.0-4.5 g/kg. Treon et al. showed fatality in rabbits exposed to 15,000 ppm for 1 hr but only some weight loss, narcosis and convulsions at 10,000 ppm (40 mg/liter).[1] Ten weeks of 5 hr/day exposures to 1162 ppm or less produced no toxicity in rabbits but 3300 ppm for the same time produced slight kidney and liver damage (See Table 2).

Due to a paucity of chronic exposure data, we instituted a one year study at concentrations of 400 and 2000 ppm on an industrial work week schedule of 6 hrs/day, 5 days/wk in mice, rats, hamsters and dogs. Data have been compiled to 20 months post exposure. The most significant change seen was an effect on growth and weight gain of the hamsters during exposure. However, within two months following exposure the animals were at control level weight. This decrease in rate of weight gain was not remarkable in male rats, and female rats at the high dose gained somewhat more than controls. The clinical chemistry of the dogs was normal.[2]

An Emergency Exposure Limit for 1 hr was determined for MCH. Rats, mice and trained dogs were exposed to various concentrations of MCH for 1 hr and observed for signs of toxicity for 28 days post exposure. From the rodent data both rats and mice appear to tolerate well an exposure of approximately 4200 ppm. The rodents indicated no loss of coordination or other sequelea, and pathology 28 days post exposure was negative. The dogs exposed to 4071 ppm produced no adverse effects during or after exposure for the 28 day observation period. Clinical chemistry was normal throughout and neurological testing revealed no exposure related effects. Until human data are available which would indicate that man is more susceptible to MCH than rodents or dogs, there is no reason to believe that a 4000 ppm concentration of MCH would prevent man from self rescue. Further, subsequent medical sequelae would appear unlikely.[3] The current TLV is 400 ppm (1600 mg/m^3) with an STEL of 500 ppm (2000 mg/m^3).[4]

RJ4 (TH Dimer) is an extremely odiferous compound but not very toxic even at air saturation levels. The acute oral LD50 in the rat is greater than 16 g/kg and the i.p. LD50 is 3.2 g/kg.[5] The compound produces respiratory tract irritation, but skin and eye irritation studies in rabbits were negative. Exposure for 6 months, 6 hrs/da, 5 da/wk at 2 mg/liter (298 ppm) produced respiratory irritation in monkeys, dogs and rats, weight depression in dogs and rats, and liver and kidney hyperplasia in rats.[6] Mutagenic potential studies using microbial assays were negative as was the mouse lymphoma test, and dominant lethal assay. Unscheduled DNA synthesis tests were vaguely positive but risk minimal. The oncogenic potential is not clear cut but if it is an oncogen, it is most certainly of low potency.[7] See Tables 3, 4 and 5 for details.

RJ5 (Shelldyne H) is also very odiferous and also not very toxic. The oral LD50 in the rat is greater than 16 g/kg while the i.p. LD50 is 3 g/kg. It also produces respiratory tract irritation, but skin and eye irritation studies were negative in the rabbit.[5] The vapor pressure of RJ5 is such that 0.15 mg/liter (20 ppm) is near saturation. Six month exposures to dogs, monkeys, rats and mice produced respiratory irritation in monkeys, rats and dogs and weight depression in dogs.[6] Mutagenic potential studies were similar to those found with RJ4.[8] See Tables 3 and 4 for details. Table 5 shows that mice held one year post 6 months exposure to 20 ppm RJ5 produced more tumors than control. Although the numbers of animals are small and the mutagenic potential experiments were negative, those data red flagged the possibility that RJ5 might be a weak tumor producer.[6]

An in-depth study is now in progress to look at the oncogenic potential of this compound. Rats, mice, hamsters and dogs are being exposed to 0.03 or 0.15 mg/liter vapor for 1 year using an industrial type regimen of 6 hr/da, 5 da/wk. At 7 months into the exposure there has been no mortality and other parameters are not biologically changed excepting a small decrease in body weight gain in male rats but not female rats, dogs or mice.[9]

JP10 is not to be considered acutely toxic by accepted toxicity standards. The single dose oral LD50 could not be determined in either rats or hamsters, both being greater than 20 ml/kg. Mice had an LD50 of 3.9 ml/k with convulsions preceding death which occurred within 48 hrs. Eye and skin irritation studies in rabbits were negative. It has shown to be a mild sensitizer in guinea pig sensitization studies. Four hour LC50's were 1221 ppm in male rats and 1194 ppm in female rats. The mouse 4 hr LC50 is approximately 900 ppm. Symptoms included eye irritation, fine tremors, prostation, and convulsions followed by death. Survivors of high level exposures had hind quarter paralysis.[10]

Emergency Exposure Limits for 10, 30 and 60 minutes have been established on the basis of mouse, rat and dog exposures, and examination of pathology (mouse and rat) and performance data in trained dogs. The recommended EEL's are 1000 ppm for 10 min, 600 ppm for 30 min and 150 ppm for 60 min.[10]

Chronic exposure studies of JP10 fuel using a 100 ppm concentration for 1 yr on an industrial exposure regimen of 6 hrs/da, 5 da/wk were instituted using rats, mice, hamsters and dogs. Mortality of the treated animals during, and 10 months following exposure, was not different from controls. Weights of male rats and hamsters showed some depression from the exposure. Female rats were not affected. Dog clinical chemistry values were not remarkable and all dogs held to date (10 months post exposure) are in good health. These animals will be maintained until June 1984 for study of oncogenic potential.[11]

The toxicity of JP4 jet fuel has been studied rather extensively. The acute oral lethal dose in the rat is greater than 8 g/kg. The 6 hr lethal concentration is greater than 38 mg/liter. It is a positive skin and eye irritant in the rabbit and produces central nervous system depression, lethargy and emesis in highly exposed individuals (Table 6).

JP4 was presented to dogs, monkeys, rats and mice for 8 months, 6 hrs/da, 5 da/wk at concentrations of either 5 mg/liter or 2.5 mg/liter. At these concentrations the fuel contained 25 ppm and 12.5 ppm benzene, respectively. Positive control animals were exposed to 25 ppm benzene using the same exposure times as the JP4 and control animals. There was increased incidence of red blood cell fragility in female dogs at the 5 mg/liter concentration and an increased incidence of chronic bronchitis in rats (Table 6).[12] The tumorigenic response was not dose related or remarkable. Further, benzene was not remarkable in that regard (Table 7). On the basis of these data, we have suggested a standard of 2.5 mg/liter as the TLV.[13, 14]

As stated before, the relative vapor pressures of the individual constituents of JP9 are extremely important in assessing the hazard associated with use. Since JP9 contains 70% JP10, 20% RJ5 and 10% MCH we have determined the vapor pressures to be approximately as follows at room temperature (70F):

MCH	9600 ppm	7 mm Hg
JP10	660 ppm	0.5 mm Hg
RJ5	66 ppm	0.05 mm Hg

If one adheres to the ACGIH 10 hr TLV for MCH of 400 ppm, the breathing zone of a worker would also contain 27 ppm of JP10 and 2.7 ppm RJ5. The exposures for JP10 and RJ5 would be at least an order of magnitude below levels necessary to see effects in laboratory animals.

Since workers performing engine tests would not likely be exposed for more than a few minutes per day if proper industrial hygiene procedures are used, and since the odors of these compounds are highly objectionable, the inhalation hazard is deemed quite low.

If hygienic procedures use MCH as the marker compound for monitoring purposes, the TLV of 400 ppm or any fraction of that figure downward should provide safe working conditions for test purposes. Further, handling of these compounds should not pose a hazard markedly different from handling JP4 fuel, when the low vapor pressure of the ramjet fuel is taken into account.

TABLE 1: PHYSICAL PROPERTIES OF RJ FUELS

	RJ4	RJ5	JP10	MCH
Empirical Formula	C12H20	C14H20	C10H16	C7H14
Molecular	164	188	136	98
Vapor Pressure (70F) mm Hg	0.354	0.025	0.50	42
Density (70F) g/ml	0.925	1.0813	0.940	0.7660

TABLE 2: METHYLCYCLOHEXANE

ACUTE TOXICITY

Oral - Rabbits	LD (Lethal Dose)	= 4.0 - 4.5 g/kg
One Hr Inhalation - Rabbits	LC (Lethal Conc Lo)	= 15,000 ppm

CHRONIC TOXICITY

Inhalation - Rabbits	LC-100 (Lethal Conc 100)	= 10,000 ppm
6 Hr/Da, 5 Da/Wk	LC-25 (Lethal Conc 25)	= 7,300 ppm
2 Wks	LC-0 (Lethal Conc 0)	= 5,600 ppm

EFFECTS

Slight kidney and liver damage at 3,300 ppm at 10 wk exposure
No kidney or liver damage at 1,162 ppm at 10 wk exposure
Eye irritation - Positive
Mucous membrane irritation - Positive

STANDARDS

Threshold Limit Value = 400 ppm (1600 mg/m^3)
Short Term Exposure Limit = 500 ppm (2000 mg/m^3)

TABLE 3: RJ-4 (TH-DIMER)

ACUTE TOXICITY

Oral	Mouse	LD Lo = 250 mg/kg
	Rat	LD 50 > 16 g/kg
Intraperitoneal	Rat	LD 50 = 3.2 (2.5 - 4.2) g/kg
4 Hr Inhalation	Rat	LC Lo = 3200 mg/m^3

EFFECTS

Highly objectionable odor
Respiratory tract irritation
Eye and skin irritation studies in rabbits - Negative

RJ-5 (SHELLDYNE H)

ACUTE TOXICITY

Oral	Rat	LC 50 > 16 g/kg
Intraperitoneal	Rat	LD 50 = 3.0 (1.9 - 4.8) g/kg
4 Hr Inhalation	Rat	LC Lo > 1969 mg/m^3

EFFECTS

Highly objectionable odor
Respiratory tract irritation
Eye and skin irritation studies in rabbits - Negative

TABLE 4: RJ4 AND RJ5 CHRONIC TOXICITY

EXPOSURE PARAMETERS
- Exposure Time = 6 months, 6 Hrs/Da, 5 Da/Wk
- Exposure Concentrations: RJ4 = 2 mg/liter (298 ppm) near saturation
  RJ5 = 0.15 mg/liter (20 ppm) near saturation
- Animals/Exposure: 8 dogs, 4 monkeys, 50 rats, 40 mice

EFFECTS
- RJ4 and RJ5 - Respiratory irritation monkeys, dogs, rats
  †Incidence bronchitis and bronchopneumonia in dogs and rats
- RJ4 - Weight depression in dogs and rats
  Kidney and liver hyperplasia in rats
- RJ5 - Weight depression in dogs

ONCOGENIC POTENTIAL
- Not clear-cut
- If oncogenic - low potency

MUTAGENIC POTENTIAL - RJ5 AND RJ4
- Microbial assay (Ames) - Negative
- Mouse lymphoma test - Negative
- Unscheduled DNA synthesis - Positive (risk minimal)
- Dominant lethal test (mouse and rat) - Negative

TABLE 5: TUMOR INCIDENCE IN MICE EXPOSED TO RJ4 AND RJ5 FOR SIX MONTHS AND HELD ONE YEAR POSTEXPOSURE

	CONTROL	RJ4	RJ5
Tumors in mice dying during postexposure period			
Sarcoma	2/5	3/6	4/6
Alveolargenic Carcinoma	1/5	0/6	0/6
Other	0/5	1/6	0/6
Tumors in all mice			
Lymphosarcoma	0/17	0/18	2/20
Alveolargenic Carcinoma	1/17	0/18	5/20
Alveolargenic Adenoma	0/17	2/18	0/20
Bronchogenic Carcinoma	0/17	0/18	1/20
Hematopoietic Sarcoma	2/17	2/18	3/20
Myelosarcoma	1/17	1/18	1/20
TOTAL	4/17	5/18	12/20

TABLE 6: JP4 FUEL

ACUTE TOXICITY

Oral	Rat	LD Lowest	>	8,000 mg/kg
	Mouse	LD Lowest	=	500 mg/kg
6 Hr inhalation	Rat	LC Lowest	>	38 mg/liter

EFFECTS
- Eye irritation - Positive
- Skin irritation - Positive

CHRONIC TOXICITY
- Exposure time = 6-8 months, 6 Hr/Da, 5 Da/Wk
- Exposure Concentrations:
  JP4 - 5.0 mg/liter (contains 25 ppm benzene)
  JP4 - 2.5 mg/liter (contains 12.5 ppm benzene)
  Benzene - 25 ppm
- Animals/Exposure:
  6 dogs, 4 monkeys, 50 rats, 40 mice

EFFECTS
- JP4 - Central nervous system depression, lethargy, emesis
  † Red blood cell fragility in female dogs at high dose
  † Incidence chronic bronchitis in rats
- Benzene - CNS depression, lethargy
- JP4 and benzene - Oncogenic response not remarkable

MUTAGENIC POTENTIAL
Microbial assay (Ames) - Negative
Mouse lymphoma - Negative
Unscheduled DNA synthesis - Non-specific damage
Dominant Lethal - Preimplantation loss (Toxic)
SUMMARY: No effect on fertility
Minimal genetic toxicity
Negative for mutagenic potential

SUGGESTED STANDARD
JP4 = 2.5 mg/liter TLV

TABLE 7: TUMOR INCIDENCE IN ANIMALS EXPOSED TO JP4 OR BENZENE FOR SIX MONTHS AND HELD ONE YEAR POSTEXPOSURE

	CONTROLS	25 PPM BENZENE	5.0 MG/L JP4	2.5 MG/L JP4
MOUSE TUMORS				
Alveolargenic Adenoma	3/19	6/17	4/16	7/21
Lymphosarcoma	0/19	1/17	1/16	2/21
Mammary Carcinoma	0/19	1/17	0/16	0/21
Hepatoma	1/19	0/17	0/16	0/21
Hematopoietic Tumors	6/19	1/17	4/16	3/21
Thyroid Carcinoma	0/19	0/17	1/16	0/21
TOTAL	10/19	9/17	10/16	12/21
RAT TUMORS				
Mammary	0/15	0/16	1/20	0/18
Thyroid Adenoma	0/15	1/16	0/20	0/18
Pancreatic Islet Cell Adenoma	0/15	1/16	0/20	0/18
TOTAL	0/15	2/16	1/20	0/18

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THE TOXICITY OF GRADE JP-5 AVIATION TURBINE FUEL, A COMPARISON BETWEEN PETROLEUM AND SHALE-DERIVED FUELS[+]

by

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SUMMARY

During the past six years the United States Navy has been active in the development of alternative hydrocarbon fuel sources for military use. Considerable interest has been focused on the large shale reserves in the Western United States. The recent Navy refinement of 100,000 barrels of shale crude provided sufficient quantities of fuels to test them for their suitability as Naval aviation fuels. In order to assess the suitability of shale-derived JP-5, it is important that its inherent toxicity be identified and that the comparative toxicity of both the shale and petroleum JP-5 be identified. The fuels were examined toxicologically from three standpoints: (1) As a liquid acute spill hazard the fuels were examined for ocular and dermal irritation potential and for skin sensitization potential. (2) Since the fuel is a complex mixture of aliphatic and aromatic hydrocarbons each exhibiting different vapor pressures, the nature of any accidental inhalation exposure will be dependent on the concentration of low boiling components in the mixture. The fuels were examined as a vapor inhalation hazard by exposing animals continuously for 90 days to vapors as high as 750 mg/m^3. Groups of animals were examined at 90 days and at the end of their normal expected lifetime. (3) The fuels were examined as a potential oncogen by histopathologic examination of the animals exposed for 90 days and held for a lifetime. Naval Medical Research Institute Toxicology Detachment activities to identify the toxicity of petroleum and shale JP-5 fuel will be reviewed.

INTRODUCTION

The Navy's principle interest in non-petroleum fossil fuel sources, or synthetic fuels, has been to determine their compatibility with naval equipment and engines, and their suitability as naval fuels[1]. Suitability includes engineering and health effects. Current interest in synthetic fuels remains focused on crude shale oil derived from surface retorting of oil shale rock from the Western United States. The Navy has participated in an interagency effort to produce and refine a large quantity of crude shale oil into Military Specification fuels for subsequent test and evaluation[1]. Recently these efforts resulted in the refinement of 80,000 bbl of shale crude. In December of 1978 shale derived JP-5 grade aviation turbine fuel was made available to the Naval Medical Research Institute/Toxicology Detachment for toxicology testing.

In order to assess the suitability of shale JP-5 it was important that the toxicity of the fuels be characterized keeping in mind the potential personnel exposure hazards ashore and aboard ship. Comparative data on the toxicity of the shale and petroleum fuels were necessary to assess engineering controls and work procedures. Unfortunately, little data was available on the dermal, ocular or inhalation toxicity of the distillate fuels used by the Navy; thus, precluding a comparative evaluation of the presently used petroleum fuels versus shale fuel. The lack of such data prompted the series of investigations whose status I will report on today.

MATERIALS

But first I would like to spend a few moments discussing the fuels used in these investigations. The first table shows the fuels studied. They were shale and petroleum

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+ Naval Medical Research and Development Command, Research Task No. MF65572001.4009. The opinions and assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large. The experiments conducted herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

TABLE 1

FUELS COMPARATIVE TOXICITY STUDY

FUEL	SOURCE
Petroleum JP-5	Air Force Aero Propulsion Laboratory Wright-Patterson Air Force Base, Ohio
Shale JP-5	100,000 bbl Paraho Crude, Sohio Refinement (1978)

JP-5 aviation turbine fuel. The shale fuel was refined from crude shale oil produced for the U. S. Navy Energy and Natural Resources Research and Development Office by the Tosco Corporation. The crude oil was refined into military specification fuel by Standard Oil Company of Ohio (Sohio). The petroleum fuel was JP-5 available at Wright-Patterson Air Force Base at the time the toxicology studies began. The military specifications allow for quite a bit of variation from one batch of fuel to another as is evident in Table 2.

TABLE 2

SOME SELECTED MILITARY SPECIFICATIONS FOR JP-5[1]

Distillation temp ^{o}C	
Initial B.P.	---
10% recovery	205
End point, max temp	290
Aromatics, vol %, max	25
Olefins, vol %, max	5
Sulfur, total wt %, max	0.4
Sulfur, mercaptan, wt %	.001
Hydrogen content, wt %	13.5
Freezing point ^{o}C, max	- 46
Density, g/ml at 15^{o}C, min	788
, max	845
Flashpoint, ^{o}C, min	60

[1] Source: MIL-T-5624K, Turbine Fuel, Aviation, Grades JP-4 and JP-5, 1 April 1976

JP-5. JP-5 is a high flash point aviation turbine fuel with a specified distillation temperature of 205^{o}C for the 10% recovery point to 290^{o}C for the end point.[2] These fuels are complex mixtures of aliphatic and aromatic hydrocarbons each exhibiting different vapor pressures. Specifications permit up to 25% aromatic hydrocarbons and 5% olefins. Substituted phenols or amines are added to inhibit oxidation.

Both fuels met military specifications with minor exceptions. The shale JP-5 had 0.21 volume percent of fuel system icing inhibitor (ethylene glycol monomethyl ether) when the military specification allows only 0.15 volume percent, and it failed the copper strip corrosion test, a subjective test for "troublesome sulfur compounds", to quote the engineers in the fuels lab.

METHODS

I'll begin the discussion of toxicity studies performed with these fuels by describing the efforts to identify ocular, dermal and sensitization potentials of the fuels. Comparative primary dermal irritation tests were performed on both fuels. The test was a patch test method conducted to measure the degree of primary dermal irritation of intact and abraded skin. Six rabbits were used for each fuel sample. The samples were in place for 24 hours. After removal of the sample patches, the test areas were scored according to the standard Draize technique.[3]

Primary eye irritation tests were performed using nine rabbits for each fuel. A 0.1 milliliter sample of fuel was applied to one eye of each rabbit. The opposite eye served as a control. Three of the treated eyes were flushed with water approximately 30 seconds after treatment with the fuel in order to assess the effectiveness of emergency treatment techniques in preventing eye injury. Irritative effects were scored at 24, 48

and 72 hours using the Draize technique.[3] Dermal sensitization tests conducted on the fuels was a modification of the Landsteiner technique[4] in that the size of the irritated area was evaluated as well as the intensity of the irritation. Twenty-four guinea pigs per fuel were given a total of seven sensitizing doses each of a 0.1 percent dilution of fuel in peanut oil over a two week period. The guinea pigs were untreated for three weeks and then given a challenge dose of fuel in peanut oil. Simultaneous injections of peanut oil alone were given as a control to each animal. Reaction to the challenge dose taking into consideration the peanut oil control reaction was recorded as a product of the length and width of the wheal multiplied by a numerical reaction score which described the reaction intensity in increasing severity.

The two major inhalation toxicity studies conducted are outlined in Table 3. The

TABLE 3

FUELS COMPARATIVE TOXICITY STUDY
INHALATION PROTOCOL SUMMARY

Laboratory:	NMRI/AFAMRL/THRU
Duration:	90-Day Continuous Vapor Inhalation
Animal Complement:	(At two concentrations per fuel)
	Rats: 75 Male and 75 Female (50 of each held for two years)
	Mice: 150 Female
	Dogs: 3 Male and 3 Female
Clinical Tests:	Body Weight, Organ Weight, Hematology, Clinical Chemistries
Histopathology:	90 Days - 42 Tissues
	2 Years - 42 Tissues

studies are being conducted at the Naval Medical Research Institute, Toxicology Detachment at Wright-Patterson Air Force Base, Ohio, utilizing Thomas Dome inhalation chambers at the Air Force Aerospace Medical Research Laboratory's Toxic Hazards Research Unit (THRU). Both fuels were studied under identical protocols. Exposures were subchronic continuous 90 day fuel vapor inhalation exposures. Inhalation chamber fuel vapors were generated by passing the fuel through a column heated to 120°F. Three animal species were employed (mice, rats and dogs) with both sexes being represented by rats and dogs. A summary of the clinical tests performed on rats and dogs at the end of the exposure period and on rats at the end of two years is shown in the table. Forty-two tissue samples are being taken from all of the animals at the 90 day point and from the mice and rats at the two year point. Tissues and blood samples taken at the two year point are intended to provide information on the long term effects of fuel exposure and also on the tumor formation potential. Table 4 indicates the inhalation chamber fuel vapor concentrations. There was one unexposed control group for each fuel tested.

TABLE 4

FUELS COMPARATIVE TOXICITY STUDY
SUMMARY OF INHALATION STUDIES AT THRU

FUEL	TEST CONCENTRATIONS
Petroleum JP-5	750 mg/m^3
	150 mg/m^3
Shale JP-5	750 mg/m^3
	150 mg/m^3

Results of the 90 day continuous studies showed an apparent dose related kidney effect in male rats. In an attempt to further define this effect another inhalation study was conducted. Two groups of 18 male and 18 female rats were exposed to shale JP-5 fuel vapors in two cubic meter chambers. The exposure concentrations were 50 mg/m^3 and 500 mg/m^3 and were 6 hour/day 5 day/week exposures for a total of 80 exposures. An equivalent unexposed group was held in an adjacent chamber to serve as a control. Table 5 outlines the study protocol. The table shows the list of clinical measurements performed. Emphasis in this study was placed on kidney function tests.

TABLE 5

SHALE JP-5, 6 HR/DAY EXPOSURE STUDY

Laboratory:	NMRI/Toxicology Detachment
Duration:	16 Weeks (80 exposures)
Concentration:	50 mg/m^3 and 500 mg/m^3
Animal Complement:	(At both JP-5 concentrations)
	Rats: 18 Male and 18 Female
Clinical Tests:	BUN, Creatinine Clearance, Albumin, SGOT, SGPT, LDH, Urine Volume, Urea Clearance, Body Weight, Glucose, Total Bilirubin
Histopathology:	Major Organs

Before I describe the results of these experiments up to this time, I think a view of the schedule and milestones of these studies is appropriate. Figure 1 depicts the time schedule we have been following. As you can see the exposure phase and the animal

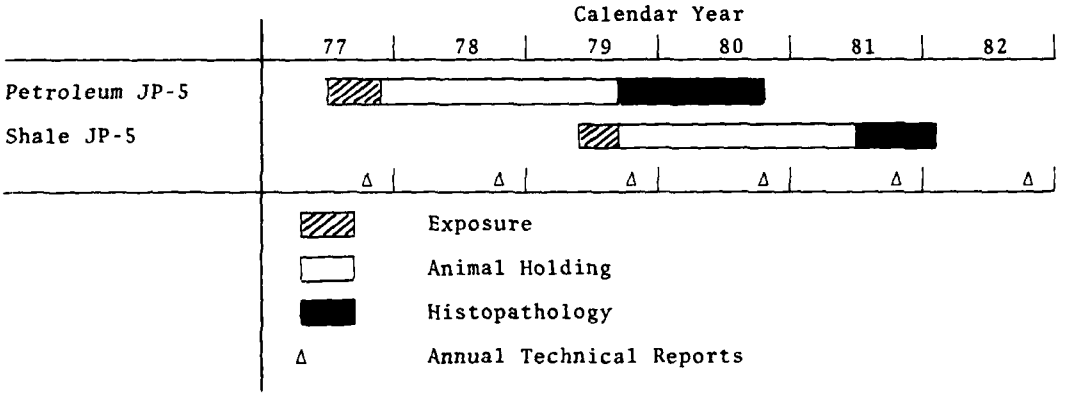

Figure 1. Fuels Comparative Toxicity Study - Schedule.

holding phase of the 90 day continuous inhalation experiments have been completed. Clinical test results at the 90 day point for each fuel are complete and results of the clinical tests from the two year, long-term follow-up of the petroleum fuel is complete. Only petroleum fuels 90 day histopathology reports are complete, however. Both Air Force and Navy demands at the Toxic Hazards Research Unit have saturated the pathology branch capability causing some delay. The target date for the fuel final reports, including the two year long-term effects follow-up, is late 1982. As you can see a very large part of the shale fuel work is incomplete at this time.

RESULTS

Since the shale fuel work is incomplete I have only partial results to report at this meeting.

Table 6 shows the results of the ocular and dermal irritation studies. Reaction to

TABLE 6

FUELS COMPARATIVE TOXICITY STUDY
SKIN AND EYE IRRITATION RESULTS

FUEL	SKIN[1]	EYE
Petroleum JP-5	None	None
Shale JP-5	None	None

[1] Intact and abraded

Skin sensitization results are summarized in Table 7. Sensitization potential is a

TABLE 7

FUELS COMPARATIVE TOXICITY STUDY
SKIN SENSITIZATION SUMMARY

FUEL	SENSITIZATION[1] POTENTIAL	SENSITIZATION[2] REACTION
Petroleum JP-5	Moderate	Mild
Shale JP-5	Slight	Mild

[1] A measure of the number of test animals exhibiting a positive reaction.

[2] A measure of the severity of reaction:

Score:	
0 - 25	No Reaction
26 - 99	Mild
100 - 200	Moderate
> 200	Severe

measure of the number of test animals exhibiting a positive reaction out of the 24 animals treated. Slight sensitization potential represents one to three animals responding and moderate represents four to 10 animals responding. Sensitization reaction is a measure of the severity of the reaction of those animals responding. Neither of the fuels tested were judged to be sensitizers according to the test criteria.

The effects of fuel vapor exposure on rat weight gain are summarized in Table 8.

TABLE 8

FUELS COMPARATIVE TOXICITY STUDY
RAT BODY WEIGHT EFFECTS SUMMARY

FUEL	MALE	FEMALE
Petroleum JP-5		
750 mg/m^3	↓ recovered	--
150 mg/m^3	↓ recovered	--
Shale JP-5		
750 mg/m^3	↓ incomplete	--
150 mg/m^3	↓ incomplete	--

Arrows express trends seen in experimental animals compared to controls

Rats exposed to the shale fuels have not been on the study long enough to see any definite health trends. The only trend we can see from this incomplete data is that male rats may be more affected by fuel vapors than female rats regardless of the fuel source. Dogs were sacrificed after the 90 day exposure and there were no apparent effects from either of the fuels during that time.

The histopathology report of rats exposed to petroleum JP-5 and sacrificed at 90 days indicated evidence for kidney injury in both exposure groups of male rats. The injury consisted of nephropathy characterized by multifocal tubular atrophy and of focal tubular necrosis at the cortico-medullary junction. Both lesions were more severe in the 750 mg/m^3 petroleum JP-5 exposure groups and appeared to be related effects, in that the more severe the nephropathy was, the more severe the tubular necrotic lesions. These changes were not seen in unexposed controls or in female rats. Neither were they seen in the dogs. Table 9 is a summary of the significant clinical measurements taken at the 90 day sacrifice. The table shows only male rat data. Statistical significance expressed here is measured between the fuel exposure group and its control and not between petroleum and shale exposure groups. The female rat data although exhibiting what appeared to be some random deviations from normal were as a whole within normal limits for the species. Male rat chemistry changes shown in this slide were considered consistent with the histopathologic findings of kidney injury.

TABLE 9

90-DAY MALE RAT CLINICAL MEASUREMENTS

	PETROLEUM JP-5			SHALE JP-5		
	Control	150_3 mg/m^3	750_3 mg/m^3	Control	150_3 mg/m^3	750_3 mg/m^3
BUN	14.9	17.0[a]	18.5[a]	17.8	17.4	20.3[b]
Creatinine	0.53	0.55	0.62[a]	0.66	0.76[b]	0.78[b]
A/G Ratio	2.5	2.3[a]	2.0[a]	1.37	1.2[b]	1.2[b]

[a] Statistically different from controls at < 0.05.

[b] Statistically different from controls at < 0.01.

At the end of the two year follow-up period, rats exposed to petroleum fuels did not exhibit increased mortality.

Results of the shale JP-5 6 hour/day exposure study appeared to confirm the observation of kidney injury apparent in both the shale and petroleum 90 day continuous exposure studies. Both groups of exposed male rats, after 20 exposure days, consumed more water than the control males. Female exposed rat water consumption was not different from female controls. Consistent with increased male rat water consumption was an increase in urine output observed in the high dose rats. Table 10 shows this increased urine output.

TABLE 10

SHALE JP-5, 6 HR/DAY EXPOSURE STUDY
RAT URINE OUTPUT

	CONTROL	50 mg/m^3	500 mg/m^3
Male			
Mean	12.2	12.0	15.4
S.E.	(.9)	(1.1)	(.9)
Female			
Mean	8.7	10.5	12.3
S.E.	(.6)	(2.1)	(.7)

The female rats were not effected. Table 11 shows the increase in plasma creatinine observed in high dose males while the female exposed rats showed levels not significantly different from their controls.

TABLE 11

SHALE JP-5, 6 HR/DAY EXPOSURE STUDY
RAT PLASMA CREATININE

	CONTROL	50 mg/m^3	500 mg/m^3
Male			
Mean	.69	.62	.84
S.E.	(.03)	(.04)	(.04)
Female			
Mean	.68	.74	.71
S.E	(.02)	(.04)	(.04)

Table 12 shows a significant decrease in the creatinine clearance of high dose male rats; however, one unusually high creatinine clearance caused an apparent elevation in the low dose males. The exposed females again exhibited no significant deviation from controls.

TABLE 12

SHALE JP-5, 6 HR/DAY EXPOSURE STUDY
RAT CREATININE CLEARANCE

	CONTROL	50 mg/m^3	500 mg/m^3
Male			
Mean	1.25	1.60*	.95
S.E.	(.07)	(.16)	(.05)
Female			
Mean	.66	.66	.74
S.E.	(.03)	(.03)	(.08)

* One animal had a high creatinine clearance of 2.65

DISCUSSION

Because the shale fuels inhalation phase of the comparative study has only recently been completed and not all of the data is available, a comparison of shale versus petroleum fuel inhalation toxicity cannot be made at this time. The irritation studies are complete and the results indicate both fuels could be considered non-irritants. Results of the skin sensitization studies indicate both were non-sensitizers.

From the results of both the 90 day continuous shale and petroleum fuel exposure study and from the shale fuel 6 hour/day study it is apparent that there is sex specific kidney injury in the male rat due to exposure to this hydrocarbon fuel. The questions of reversibility and of long term effects will not be answered until review of the data, particularly the histopathologic data, is complete at the termination of lifetime holding of the 90 day continuously exposed rats.

Although it may be premature to speak about the toxicity of a material that chemically may vary from batch to batch or change within a batch upon storage, it has been our belief that at the very least the work will serve as a benchmark from which to compare later shale fuels. At the very best we may hope for a clean bill of health for an important alternate fuel.

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Differential Effects of Hydrazine Compounds on B- and T-Cell Immune Function

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Summary

The immunotoxic effects of four hydrazine compounds were evaluated by adding them to lymphocytes in the lymphocyte blast transformation (LBT) assay. 1,1-dimethylhydrazine (UDMH) caused an enhancement of the LBT response of murine splenocytes to the B-cell mitogen lipopolysaccharide (LPS) at concentrations of 10-25 ppm. It also enhanced the LBT response of feline peripheral mononuclear cells (PMC) to the T-cell mitogens concanavalin A (con A) and pokeweed; and enhanced the LBT response of human PMC to con A, within the same concentration range. Higher concentrations suppressed the LBT response.

The UDMH was also injected intraperitoneally into mice, which were then evaluated for immune function by the LBT assay, and the Jerne plaque assay. Certain dose regimens of UDMH resulted in an enhanced LBT response of splenocytes to con A, as well as an enhanced number of antibody-secreting cells against sheep red blood cells.

1,2-dimethylhydrazine (SDMH) exerted an effect similar to that of UDMH when added to splenocytes in the LBT assay, causing an enhancement of the LBT response to LPS at low concentrations, then a suppression at higher concentrations. Hydrazine (Hz) and monomethylhydrazine (MMH) suppressed the LBT response of splenocytes to both con A and LPS, but the con A response was significantly more suppressed than the LPS response at most concentrations tested.

These results suggest that UDMH and SDMH abrogate suppressor cell function, and that Hz and MMH suppress T-cell function (cell-mediated immunity) more than B-cell function (humoral immunity).

As research in the field of immunotoxicology expands, researchers are finding that numerous chemical compounds are capable of adversely affecting the immune system. These effects often occur at subtoxic or subcarcinogenic exposure levels. Environmentalists are concerned with industrial pollutants and residues which may cause subtle immune dysfunctions after short- or long-term exposure. Chemicals which have been shown experimentally and/or epidemiologically to be immunosuppressive include hexachlorobenzene (1), polychlorinated biphenyls (1,2,3), polybrominated biphenyls (4), tetrachlorobenzo-p-dioxin (TCDD) (5), lead (6), cadmium (6), mercury (6), and methylnitrosourea (7). Some of these compounds such as TCDD are more toxic to the cell-mediated (T-cell) branch of the immune system (5), others are thought to suppress the humoral (B-cell) system more.

Previous research reports suggest that the T-cell subset of immunoregulatory ("suppressor T") cells may be susceptible to many commonly used therapeutic agents, such as cyclophosphamide (8) and methyldopa (9). Other drugs are frequently reported to be associated with a "lupus erythematosus-like syndrome," (10) including phenylbutazone, hydralazine, procainamide, isoniazid, and oral contraceptives. Further study of other drugs and chemicals will undoubtedly reveal a vast number of agents which specifically suppress T-cell or B-cell subsets.

The purpose of the studies reported here was to evaluate the effects of various hydrazine compounds (used in rocket fuels) on T-cell and B-cell function as assessed by lymphocyte blast transformation (LBT) response and plaque-forming cell response.

Materials and Methods

In Vitro Experiments:

Spleen cells:

Swiss outbred mice weighing 20-30 gms were sacrificed by cervical dislocation, and the spleens aseptically removed and placed in Hank's balanced salt solution (HBSS: Grand Island Biological Co., Grand Island, NY). Sixteen to 24 mice (male and female) were used to test each chemical. The spleens were separately teased, strained through #60 wire mesh, and aspirated several times through a 25 g needle. The red cells were lysed with an ammonium chloride (0.15 M) lysing solution, and the white cells were then washed and suspended in cell culture medium (CCM) consisting of RPMI 1640 (Grand Island Biological Co., Grand Island, NY), supplemented with 2 X 10^{-5} M 2-mercaptoethanol, 10% fetal bovine serum (Sterile Systems, Inc., Logan, UT), and 1% antibiotic solution. The splenocytes were counted and diluted to 10^{6} lymphocytes/ml. Viability was 97-100%. Percent of lymphocytes ranged from 85-99% (other cells being macrophages or neutrophils).

Feline and human cells:

Heparinized blood was drawn from normal adult specific-pathogen-free cats or from healthy human volunteers. Peripheral mononuclear cells (PMC) were separated by centrifugation through a ficoll-diatrizoate (Ficoll-Paque, Pharmacia Fine Chemicals, Piscataway, NJ) gradient, as described by Cockerell et al. (11). The washed PMC were suspended at 1 X 10^6 cells/ml in RPMI 1640 with either 20% pooled feline serum (feline PMC) or 5% human AB serum (Grand Island Biological Co., Grand Island, NY) (human PMC).

Lymphocyte blast transformation assay:

Microtiter plates (CoStar, Cambridge, MA) were filled with 0.1 ml cell suspension (10^5 lymphocytes) per well. Concanavalin A (con A) (Sigma Chemical Co., St. Louis, MO) and lipopolysaccharide (LPS: derived from E. coli 0127:B8; Difco Laboratories, Detroit, MI) were added to murine splenocytes in 10 µl aliquots (0.2 µg/well of con A and 10 µg/well of LPS). Con A (10 µg/well) and pokeweed mitogen (PWM) (Grand Island Biological Co., Grand Island, NY) (4 µg/well) were added to feline cells; and con A (10 µg/well) and phytohemagglutinin (PHA) (Difco Laboratories, Detroit, MI) (1.2 µg/well) were added to human cells. Quadruplicate wells were set up for each mitogen and media control or chemical combination (see below). Cultures were incubated at 37°C in a 5% CO_2 humidified incubator for 54 hrs, then pulsed with 0.5 µCi/well of ^{3}H-TdR (New England Nuclear, Boston, MA) and harvested 18 hrs later using a multiple automated sample harvester (Otto Hiller Co., Madison, WI). The glass fiber filter disks containing the harvested cells were dried and placed in vials with scintillation cocktail. Counts per minute (cpm) were obtained using a liquid scintillation counter (Packard Tricarb Model 3375, Packard Instrument Co., Downers Grove, IL).

Chemical Treatment:

Hydrazine (Hz), monomethylhydrazine (MMH), and 1,1-dimethylhydrazine (UDMH) were obtained in liquid form (Aldrich Chemical Co., Inc., Milwaukee, WI). They were diluted with CCM and added to the cell culture at final concentrations ranging from 0.1-30 ppm for Hz and MMH, and 10-200 ppm for UDMH. 1,2dimethylhydrazine (SDMH) was also obtained in liquid form (Aldrich Chemical Co., Inc., Milwaukee, WI) and further purified to crystalline form by Dr. Donald Witiak of the Dept. of Pharmacy, OSU. A stock solution was made by dissolving SDMH in 0.1 N HCl at a concentration of 10 mg/ml. It was then further diluted in CCM to final concentrations of 5-250 µg/ml.

Viability

Cell viability was determined by the trypan blue dye exclusion test. One hundred cells were counted for each determination, and the results expressed as

$$\frac{\text{\# viable cells}}{\text{\# total cells}} \times 100.$$

Expression of results and statistics:

Corrected cpm values were determined as follows: (cpm of mitogen-stimulated cultures)-(cpm of cultures with media alone). Percent of control response was determined by: (corrected cpm of chemically treated cultures) ÷ (corrected cpm of untreated cultures) X 100. Significant differences between groups were determined by the paired student t-test.

In vivo UDMH experiments:

Swiss outbred mice were injected intraperitoneally with varying doses and schedules of UDMH or phosphate buffered saline (PBS). Four days before sacrifice, some were immunized intravenously with sheep red blood cells (SRBC) (0.2 ml of a 10% suspension). After sacrifice, spleen cells were harvested as described and then lymphocyte function was assayed by the LBT method as described, except without addition of chemicals to the cultures, or by a modified Jerne plaque assay (see below).

Jerne plaque assay:

The agar-free slide modification described by Cunningham et al. (12) was used. Direct and/or indirect plaque forming cells (PFC) were counted and expressed as number of PFC/10^6 spleen cells.

Results

Murine splenocyte LBT response; in vitro incubation:

Hydrazine caused a dose-related suppression of the LBT response to both LPS and con A starting at a concentration of 0.1 ppm (Fig. 1). The response to con A was significantly more suppressed than the response to LPS at doses of 0.1 and 1 to 20 ppm. The responses ranged from 27.7 ± 8.7% (standard error of the mean) of the control response at 30 ppm ($p < .001$) to 85.4 ± 9.3% at 0.1 ppm ($p < .001$) for con A, and 34.5 ± 10.0% ($p < .001$) to 95.2 ± 5.3 ($p < .025$) for LPS. Cell viability was slightly lower than control in the LPS-stimulated cultures at 20 and 30 ppm (Table 1).

The suppressive effects of MMH were very similar to those of Hz (Fig. 1). The response to con A ranged from 42.1 ± 8.5% of the control response at 20 ppm MMH to 91.1 ± 4.8% at 0.1 ppm. The response to LPS ranged from 67.8 ± 9.5% at 20 ppm to 95.8 ± 5.8 at 0.1 ppm. The response to LPS was significantly higher than the response to con A at 1 to 20 ppm. Cell viability was slightly decreased at 20 ppm MMH in the LPS-stimulated cultures (Table 1).

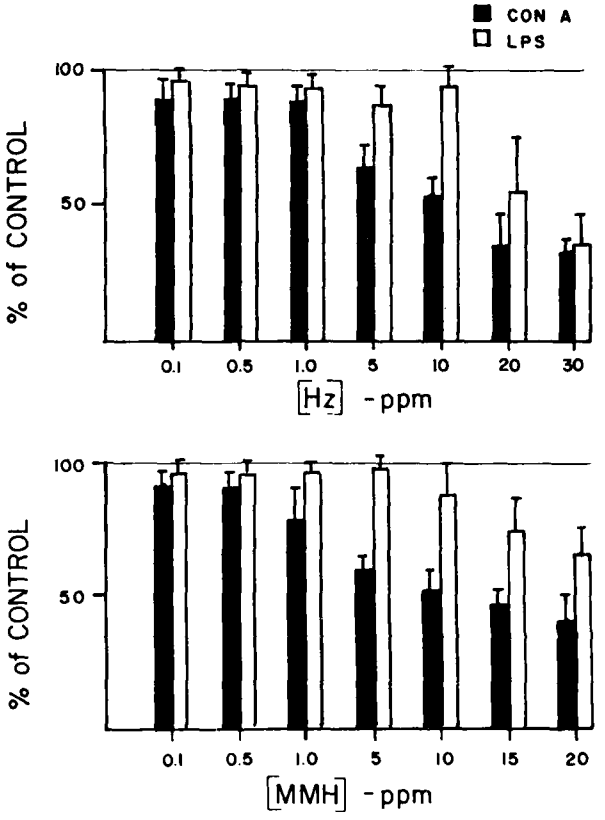

Figure 1. Effects of Incubation with Hz or MMH on the LBT Response of Murine Splenocytes to con A and LPS. Expressed as Percent of Control (Untreated Cell) Response.

Unsymmetrical dimethylhydrazine caused a significant suppression of the response to con A at concentrations of 25 ppm (89.5 ± 5.6% of control response ($p < .001$)) to 150 ppm (12.9 ± 7.3 of control response; $p < .001$) (Fig. 2). The LBT response to LPS was significantly enhanced at 10 ppm (113.9 ± 9.5% of control response; $p < .001$) and 25 ppm (112.2 ± 10.9% of control response; $p < .01$), and then suppressed at concentrations of 100 to 150 ppm (46.0 ± 6.9; $p < .001$ to 12.9 ± 7.3%; $p < .001$, respectively) (Fig. 2). The viability of con A-stimulated cells was significantly lower than the control at 50-150 ppm while the viability of LPS-stimulated cells was decreased at 150 ppm (Table 1).

The effects of SDMH on the LBT response to con A and LPS were similar to those of UDMH (Fig. 2). The response to con A was significantly suppressed starting at 25 µg/ml (87.5 ± 9.0% of control response; $p < .005$) while the response to LPS was significantly enhanced at 5 to 50 µg/ml (highest at 25 µg/ml; 119.7 ± 9.1% of control response; $p < .001$). The response to LPS became lower than control at 75 µg/ml (83.3 ± 13.0%; $p < .025$).Cell viability was decreased at 150 µg/ml in the LPS-stimulated cultures, and at 50-150 µg/ml in the LPS-stimulated cultures.

Feline and human PMC LBT response:

Preliminary experiments were performed to determine the effects of UDMH on the LBT response of human and feline PMC. The feline PMC showed a markedly enhanced LBT response to PWM which was highest at 50 ppm UDMH (Fig. 3)(164.3 ± 29.7% of control response; $p < .025$). There was also a slightly enhanced response to con A at 10-50 ppm UDMH, which peaked at 25 ppm (115.6 ± 23.9; NS). The LBT response to PWM dropped below the control value at 150 ppm UDMH and to con A at 75 ppm. The response to con A of the human PMC was

similarly affected by UDMH (Fig. 3), showing an enhanced response which peaked at 50 ppm (110.3 ± 2.8; NS), then falling below the control response at 150 ppm. The UDMH had little effect on the human LBT response to PHA, showing only a slight decrease at the highest UDMH concentration tested, 200 ppm (88.1 ± 8.7% of control response; NS) (Fig. 3).

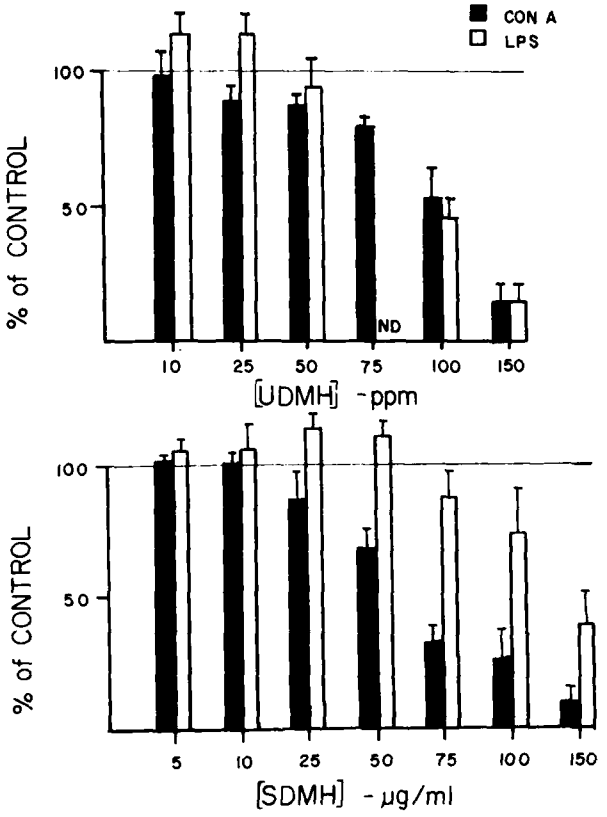

Figure 2. Effects of Incubation with UDMH or SDMH on the LBT Response of Murine Splenocytes to con A and LPS. Expressed as Percent of Control (Untreated Cell) Response.

Table 1. Viabilities of splenocytes treated with Hz, MMH, UDMH, or SDMH.

CPD	Dose (ppm)	Con A	P	LPS	P	Media	P
Hz	0 (control	80.5 ± 5.1		90.5 ± 1.0		77.0 ± 1.7	
	1	90.5 ± 2.2	NS	91.5 ± 1.7	NS	83.0 ± 1.9	p<.05
	10	79.0 ± 1.2	NS	85.0 ± 4.7	NS	77.5 ± 1.3	NS
	20	76.0 ± 4.5	NS	75.0 ± 5.4	p<.025	69.5 ± 4.3	NS
MMH	0 (control)	87.0 ± 4.7		88.0 ± 2.7		78.0 ± 3.6	
	.01	90.5 ± 2.2	NS	94.5 ± 1.9	p<.05	72.0 ± 4.2	NS
	5	89.5 ± 3.9	NS	94.5 ± 1.7	p<.05	68.5 ± 4.0	NS
	20	87.5 ± 3.9	NS	79.5 ± 2.6	p<.05	85.0 ± 1.9	NS
UDMH	0 (control)	86.5 ± 2.2		91.5 ± 1.5		80.0 ± 5.9	
	10	80.5 ± 4.6	NS	95.5 ± 0.5	p<.025	76.0 ± 4.1	NS
	50	67.0 ± 6.5	p<.025	92.0 ± 2.5	NS	66.0 ± 5.6	NS
	100	68.3 ± 8.0	p<.05	89.0 ± 1.9	NS	58.5 ± 2.1	p<.01
	150	68.5 ± 3.9	p<.005	78.0 ± 3.5	p<.01	65.0 ± 7.0	NS
SDMH	0 (control)	82.6 ± 5.4		87.4 ± 2.0		82.0 ± 2.8	
	5	86.0 ± 3.0	NS	87.4 ± 2.9	NS	81.7 ± 4.7	NS
	10	84.6 ± 2.9	NS	86.6 ± 2.8	NS	80.0 ± 3.6	NS
	25	86.3 ± 4.1	NS	89.4 ± 3.3	NS	78.9 ± 4.0	NS
	50	77.4 ± 1.9	NS	78.6 ± 4.8	p<.025	77.2 ± 3.6	NS
	75	81.0 ± 1.3	NS	77.6 ± 3.4	p<.01	81.2 ± 2.4	NS
	100	74.2 ± 5.3	NS	63.8 ± 5.4	p<.001	75.2 ± 2.8	p<.05
	150	70.2 ± 3.6	p<.05	65.6 ± 4.0	p<.001		

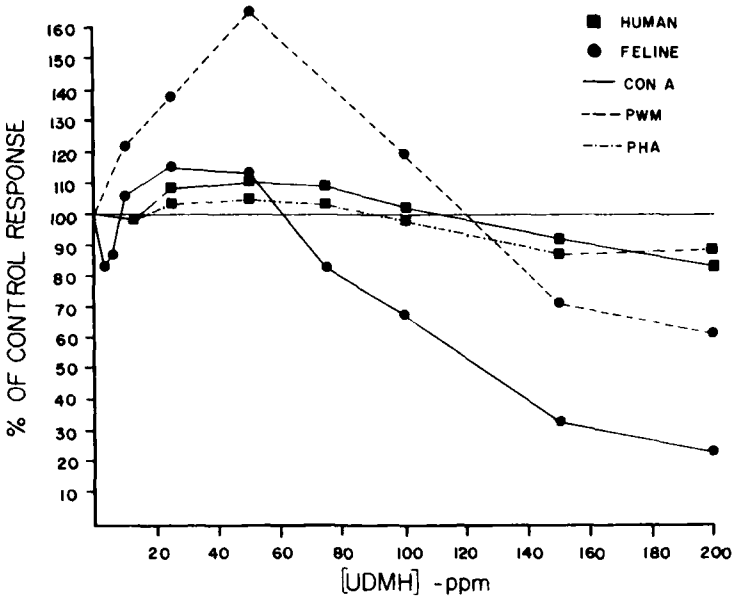

Figure 3. Effects of Incubation with UDMH on the LBT Response of Feline PMC to con A and PWM, and of Human PMC to con A and PHA. Expressed as Percent of Control (Untreated Cell) Response.

In vivo UDMH experiments:

LBT assay: Short-term low dose treatment (e.g. 50 mg/kg UDMH daily for 3 days or 15 mg/kg single dose) resulted in an enhanced LBT response of the spleen cells to con A (up to 1-1/2 times the response of cells from untreated mice) (Table 2), but did not affect the LBT response to LPS. Higher doses of UDMH (single dose of 150 mg/kg) caused a decreased response to LPS (Table 2).

Table 2. Effects of Intraperitoneal UDMH injections on the LBT response of spleen cells to mitogens.[a]

Dose UDMH	Mitogen	UDMH-treated (CPM ± sem)	Untreated control (CPM ± sem)	P-Value
15 mg/kg one dose	con A	54894 ± 11497	36741 ± 13680	$p < .025$
50 mg/kg/day 3 days	con A	59085 ± 7454	47701 ± 4496	$p < .05$
150 mg/kg one dose	LPS	69206 ± 3696	76626 ± 9038	$p < .05$

[a] Mice were sacrificed 24 hours after the last UDMH injection. Treated and control groups each consisted of 8 mice (4 male, 4 female).

Jerne plaque assay: Female mice treated with 100 mg/kg daily for eight days (with SRBC immunization on the fourth day of treatment) showed an increased number of direct PFC (IgM-forming cells) (Table 3). In similar experiment, treated male exhibited an increased number of indirect PFC (IgG-forming cells), with no difference in numbers of direct PFC (Table 3).

Table 3. Effects of intraperitoneal UDMH injections on PFC response to SRBC immunization.

	PFC/10^6 spleen cells ($X10^3$)		
	UDMH-treated	Control	P-Value
Exp 1[a]			
Male-direct	1.41 ± 0.47	1.80 ± 0.52	NS
Female-direct	3.71 ± 0.32	2.81 ± 0.32	$p < .005$
Exp. 2[b]			
Male-direct	0.55 ± 0.14	0.51 ± 0.11	NS
Female-direct	0.18 ± 0.11	0.22 ± 0.10	NS
Male-indirect	2.05 ± 0.53	1.41 ± 0.18	$p < .05$
Female-indirect	0.86 ± 0.18	0.67 ± 0.17	NS

[a] Mice (3/group) were injected i.p. with 100 mg/kg UDMH for 4 days, immunized i.v. with 0.2 ml of a 10% SRBC suspension, treated 4 more days with UDMH, and sacrificed.

[b] Mice (3/group) were injected i.p. with 100 mg/kg UDMH for 4 days, immunized i.v. with 0.2 ml of a 10% SRBC suspension, rested two days, then treated 3 more days with UDMH before sacrifice.

Discussion

These studies clearly show that hydrazine compounds exert a differential effect on in vitro mitogen-induced LBT responses of murine splenocytes. The response to the T-cell mitogen con A was significantly more suppressed by MMH, Hz, and SDMH than the response to the B-cell mitogen LPS. The UDMH and SDMH caused an enhancement of the B-cell response to LPS at low concentrations, then a suppression of both T-cell and B-cell responses at higher concentrations.

The enhancement of the LPS response as a result of incubation with UDMH and SDMH could be explained by a selective inhibition of suppressor T-cell function at lower concentrations of UDMH and SDMH. As the concentrations increase, effector T-cell functions, as well as B-cell functions, decrease.

The possibility of a selective decrease in suppressor T-cell function induced by UDMH is supported by the results of preliminary in vivo experiments in which spleen cells from mice treated with UDMH showed an enhanced LBT response to con A or increased numbers of PFC in the Jerne plaque assay, compared to untreated mice. Experiments are currently underway involving long-term treatment of mice with different doses of UDMH after which LBT response and PFC response will be measured. In addition, planned experiments involving UDMH effects on in vitro suppressor cell function assays and identification of suppressor cell subsets by membrane markers in UDMH-exposed lymphocyte cultures, will hopefully determine if UDMH is indeed specifically inhibiting suppressor cell function at certain concentrations.

Incubation with UDMH resulted in a similar enhancing effect on the LBT response of lymphocytes from other species. Feline PMC showed an enhanced response to both con A and PWM which are T-cell mitogens in the cat. Human PMC also responded more to the T-cell mitogens con A and PHA during UDMH exposure. The enhanced response to T-cell mitogens was not seen with murine splenocytes. This difference in response to T-cell mitogens may reflect the different sources of lymphocytes (PMC from cats and humans, splenocytes from mice).

Similar enhancing effects of various immune parameters have been reported for other environmental pollutants at low doses. Holt et al. (13) noted that short-term exposure of mice to NO or NO_2 gas resulted in enhanced LBT response to PHA; enhanced graft-versus-host reaction; and enhanced antibody response to T-dependent antigens, as well as increased serum immunoglobulin levels. Long-term exposure, however, caused suppression of these parameters. In another experiment, Sharma and Gehring (14) noted an enhanced background response in cultured splenic lymphocytes from mice treated with low doses of TCDD. These mice also showed increased immunoglobulin levels, prompting the authors to suggest "the hypothesis of an auto-immune response" as a result of TCDD treatment. Again, higher doses of TCDD suppressed immunoglobulin levels and mitogen responsiveness.

As mentioned earlier, a selective loss of suppressor cell function in the intact animal is associated primarily with autoimmune disorders such as rheumatoid arthritis, autoimmune hemolytic anemia, and systemic lupus erythematosus. A substituted hydrazine compound, 1-hydrazinophthalazine or hydralazine (used in clinical medicine to control hypertension) has long been associated with side effects of acute rheumatoid state and/or disseminated lupus erythematosus (15), including the presence of antinuclear antibodies. With the recent development of methods to identify the presence and function of the suppressor T-cell subset, several drugs and compounds have already been found which specifically inhibit suppressor T-cell function, and undoubtedly, many more will be found in the future.

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HYDRAZINE EFFECTS ON VERTEBRATE CELLS IN VITRO

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This study was designed to elucidate the cellular effects of hydrazine on four established tissue culture vertebrate cell lines (rat kangaroo kidney, Xenopus toad kieney, human diploid fibroblast and Chinese hamster cells) and primary cultures of neonatal rat myocardial cells. Cells were exposed to hydrazine in various concentrations (0.001 mM to 10 mM) for varying time periods. The resulting growth and morphological data revealed a possible site of hydrazine action.

In all cell lines tested, population growth was depressed by low concentrations of hydrazine (0.01 mM to 0.1 mM). Cell growth was initially depressed, but it eventually returned to normal log phase growth even when fresh hydrazine was added to the culture medium. At higher concentrations (0.5 mM to 2.0 mM), hydrazine was lethal. Most cell types first showed population growth depression at 0.01 mM hydrazine, but the lethal concentration varied with the cell type. Cultures treated with hydrazine yielded a significantly higher number of giant, multinucleated cells. Autoradiography studies employing ^{3}H-thymidine confirmed that the large, multinucleated cells resulted from cell fusion.

The increase in cell fusion in hydrazine treated cell cultures implicated the cell surface as a possible target site. Scanning electron microscopy confirmed concentration related surface differences between control and hydrazine treated cells. Further membrane studies examining the effects of hydrazine on the contractile and intercellular spontaneous electrical activity of myocardial cells in culture indicated that hydrazine also altered these membrane-related activities in a concentration and time dependent manner.

List of abbreviations: M=molar, mM=millimolar, ml=milliliter, min=minute(s), x g=times gravity, mg=milligrams, N=normal, Fig.=figure, hr=hour(s), μCi=micro curies, SEM=Scanning Electron Microscopy, V=volts.

INTRODUCTION

Hydrazine (NH_2NH_2) is a hydroscopic, highly polar reducing agent (Raphaelian, 1966). This reactive compound, described as the ammono analog of hydrogen peroxide, can be converted to a variety of widely used alkyl derivatives. Both hydrazine and its derivatives are used extensively in the production of photographic developers, agricultural chemicals, and pharmaceutical products. The use of hydrazine both as an oxygen scavenger in industry and as a major component in high energy rocket fuel cells constitutes its major commercial uses. As a result of these applications, hydrazine and its derivatives are becoming more prevalent in the environment, and their use has been criticized as a source of biological hazard.

Previous studies have detailed the hazardous effects of hydrazine and related derivatives. Hydrazine is known to effect pyrimidine-related mutations in DNA (Brown et al., 1966; Brown, 1967; Gupta and Grover, 1970; Kak and Kaul, 1975), and it is easily derivatized into a number of detrimental agents which act as both toxins and carcinogens. The most studied of these agents include the toxins hydralazine, which interfers with smooth muscle contraction (McLean et al., 1978), phenyl hydrazine, a hemolytic agent including anemia and Heinz body formation (Jain et al., 1978) and monomethyl hydrazine, a metabolic inhibitor (Dost et al., 1976). A carcinogenic relative of the latter derivative, dimethyl hydrazine, has produced both colon and blood vessel tumors in several laboratory animals (Toth and Wilson, 1971; Toth et al., 1976; Mak and Chong, 1978; Barkla and Tutton, 1978). More specifically, some of these effects are produced in isolated cellular components only under specific conditions of treatment (Brown, 1967; Kak and Kaul, 1975). The importance of these studies may be underestimated or misinterpreted without a basic understanding of the impact such compounds have on a wide variety of cell types when tested under controlled conditions.

The present study investigates the basic cellular responses of diverse vertebrate cell types in vitro to the compound hydrazine. The results implicate the cell membrane as one of the major targets of hydrazine action.

METHODS

Maintenance of Cell Cultures

The PTK_2 cell line, which was derived from normal adult male kidney of Potorous tridactylus, was obtained from the American Type Culture Collection (CCL 56). Cells were grown in minimum essential medium (Eagle) with Earle's salts supplemented with 0.085% sodium bicarbonate, 10% heat inactivated fetal calf serum and 0.011% pyruvic acid. Cultures were maintained at 37°C in a 5% CO_2, 95% air atmosphere in Falcon T-75 plastic flasks. Cells were enzymatically detached from the flasks as follows: the supernatant overlying the cell monolayer was aspirated from the T-75 flask and replaced with 4 ml of an enzymatic solution

(0.25% Pancreatin, 0.1% EDTA in a balanced salt solution, pH 7.0). Cells detached from the substrate during incubation (6 min., 37°C) and light pasteur pipetting. The enzymatic solution was diluted out with 5 ml of medium, and the cells were pelleted from the suspension (5 min., 200 x g). The resulting supernatant was discarded, and the cells were diluted in fresh medium for plating into either T-75 flasks (4×10^5 cells/ml in 10 ml of medium/flask) for growth curves or into Rose multipurpose chambers (50×10^3 cells/ml, Berns et al., 1972) for microscopic evaluation.

The A6 cell line (American Type Culture Collection, CCL 102), derived from primary culture of normal male toad (Xenopus laevis) kidney, was maintained in Eagle's minimum essential medium (Hank's salts) fortified with 10% heat inactivated fetal calf serum, penicillin (100,000 I.U./liter) and streptomycin sulfate (0.0714 g/liter) in a 5% CO_2, 95% air atmosphere at room temperature. For weekly subculturing and experimental set-ups, the cells were enzymatically removed from the flasks as previously described (4 min incubation at room temperature) and plated into T-75 flasks for growth curves (4×10^5 cells/flask in 10 ml of medium) or into Rose chambers (75×10^3 cells/ml) for microscopic evaluation.

Human diploid embryonic lung cells (WI38 were purchased from the American Type Culture Collection (CCL 75). These cells were maintained in Eagle's basal medium (Earle's salts) with 10% un-inactivated fetal calf serum in a 5% CO_2, 95% air atmosphere. For weekly subculturing and growth curve experiments, the cells were enzymatically detached from the flask as previously described with the following modification: the cells were incubated 5 min at 37°C in an enzymatic solution consisting of 0.25% trypsin in a balanced salt solution, pH 7.0.

Chinese hamster cells (CH), also referred to as the MC-3 line, were generously provided by Dr. Joe Gray (Lawrence Livermore Laboratory, Livermore, CA). They were maintained in minimum essential medium (Earle's salts) supplemented with 15% heat inactivated fetal calf serum, 4.6% NCTC-135 (GIBCO, Grand Island, NY) and 60 mg/liter gentamycin sulfate (Schering Corp., Kenilworth, NJ) in a 10% CO_2, 90% air atmosphere at 37°C in T-25 flasks (Falcon). After enzymatic detachment from flasks (incubated 5 min, 37°C), these cells were plated in Falcon T-25 flasks at 2.5×10^4 cells/flask in 5 ml medium for growth experiments.

Hydrazine Stock Solutions

Hydrazine (m.w. 32.05, anhydrous, 97%) was supplied through the courtesy of Dr. Ronald Shank (Univ. of Ca., Irvine). Stock solutions of 100 mM and 10 mM hydrazine were prepared by dilution into 0.01 N HCl. The stock solutions were diluted from 50 to 1,000 fold in tissue culture medium just prior to application on the cultures. Control cultures received a corresponding amount of 0.01 N HCl without hydrazine in the medium. In the quantities used, the addition of 0.01 N HCl with or without hydrazine did not significantly alter the pH of the medium.

Growth Response Curves

A. Normal Growth Curves

To study the growth effects of hydrazine on a particular cell line, the specified quantity of cells were plated out in Falcon T-75 flasks in tissue culture medium. Sufficient flasks were prepared such that two flasks were counted to determine each data point for a given hydrazine concentration. At the specified times (indicated by arrows, Figure 1), two flasks from the entire population were harvested as follows: the cells in a flask were examined by inverted phase microscopy, washed with fresh medium to remove debris, enzymatically removed from the flask as previously described, and counted and sized by a Coulter Counter-Channelyzer (model ZBi, Coulter Electronics). At this time, the medium in all the remaining flasks was replaced with fresh medium and supplemented with the appropriate concentration of fresh hydrazine stock solution. Control cultures received fresh medium and a corresponding aliquot of 0.01 N HCl without hydrazine. At each subsequent point of harvest, two flasks of cells representing each hydrazine concentration were similarly harvested, and the medium in the remaining flasks was replenished again with fresh medium containing the appropriate concentration of fresh hydrazine. The medium was changed at different intervals in the various experimental schemes. The particular timing for each experiment is depicted in the figures.

B. Time Growth Curves

To determine how the length of hydrazine exposure might correlate to hydrazine-induced effects on cell cultures, both PTK_2 cells and A-6 cells were treated to a growth suppressive concentration of hydrazine (1.0 mM) for varying lengths of time. In this experiment, the cells were set up and harvested in the previously noted manner incorporating the following modifications: at the indicated initial exposure time (Fig. 2, arrows at day 2), an aliquot of hydrazine stock solution was introduced into each flask of established cell cultures containing freshly replenished medium. The flasks of cultures were then divided into sets which would receive exposures to hydrazine for 24 hours, 96 hours, or throughout the duration of the experiment (continuous). In the A6 cell line, the 24 hour flasks received just one exposure to hydrazine (at the time point indicated); the 96 hour exposure received fresh hydrazine after medium changes at the initial time point (arrow, Fig. 2), day 3, and day 5; the continuous exposure group received fresh hydrazine after medium changes at the initial time point (arrow, Fig. 2), day 3, day 5, and day 8. The PTK_2 cells received the same schedule of treatment except the exposure time was at day 6 rather than at day 5, as indicated for the A6 cells. Beyond the designated time of exposure, each flask in a set was replenished with fresh medium and an equivalent aliquot of 0.01 NHCl without hydrazine.

The control flasks received fresh medium containing 0.01N HCl at all time points indicated in Fig. 2.

C. Selection Growth Curves

Flasks of A6 cells were plated out as previously described. Forty-eight hours after establishing cell cultures, the medium in the flasks was discarded, replenished with fresh medium, and supplemented with an aliquot of stock hydrazine solution to yield a final concentration of 1.0 mM. This procedure was repeated at 48 hour intervals up to the 10th day when the cultures approached confluency. At this point, these hydrazine "selected" cells were harvested and plated out in flasks for growth analysis as previously described. The above procedure was then repeated with the following modifications: both flasks of "selected" A6 cells and parallel flasks of non-selected, "naive" A6 cells were treated as described with 1.0 mM hydrazine at the points indicated in Fig. 3 throughout the experiment. Parallel flasks of "selected" cells receiving a corresponding aliquot of 0.01N HCl without hydrazine served as a control.

PTK_2 Large Cell Analysis

PTK_2 cells were established in Rose chamber cultures as previously described except that experimental chambers were plated out in medium containing 1.0 mM hydrazine. The chambers received fresh medium (control set) and medium containing 1.0 mM hydrazine (experimental set) at 48 hr and 96 hr post plating. Each of three chambers constituting a set was then photographed at twenty random sites (40X, Kodak Panatomic X film, ASA 32) with a Zeiss photomicroscope. From the photographs, the frequency of multinucleation occurring in both the control and experimental population was tabulated. Statistical differences between the two populations were determined by the two tailed t-test with at least 95% confidence.

PTK_2 Cell Fusion Assay

Two sets of duplicate T-25 flasks were set up with 10^5 PTK_2 cells in 5 ml of medium. One set of duplicate flasks received 1.0 mM hydrazine 24 hr after plating, and the second set served as a control. Fresh medium was substituted 36 hr later with one flask from each set receiving ^{3}H-thymidine (2μCi/ml, ICN Pharmaceuticals, Irvine, CA). The cells in each set of flasks were pulsed for 8 hr, rinsed, enzymatically removed and co-suspended into a a common culture containing both ^{3}H-thymidine-labeled and regular cells. Each mixed culture set was plated into two Rose chambers (40 x 10^3 cells/ml medium) with experimental chambers again receiving 1.0 mM hydrazine. After 12 hr, glass coverslips with the adherent cells were fixed in medium containing 2% diglutaralehyde (EM grade, Polysciences, Warrington, PA) for 1 hr at room temperature and overnight at 4°C. The coverslips were then washed in phosphate buffered saline and mounted on glass microscope slides. Autoradiography was performed in total darkness as follows: the slides were dipped into a 33% aqueous solution of Ilford nuclear research emulsion type L-4 (batch PL-729, CIBA-GEIGY Co., Ilford, Ltd., Basidon Essex, Essex, England), dried for 1 hr at room temperature and stored in a dessicant-containing, light tight box (4°C). One week later, the slides were developed using Kodak D-19 developer (5 min), 1% aqueous acetic acid stop (15 sec), Kodak fixer (5 min) and water rinse (20 min); all solutions were used at 18°C. The autoradiographs were stained in a filtered, saturated aqueous methylene blue solution (15 min) and destained with water. Permanent mounts were made of the slides after a series of ethanol dehydrations.

Electron Microscopy

Xenopus cells (75 x 10^3 cells/ml) were plated into Rose chambers in medium containing either 0.01N HCl with hydrazine (final experimental culture hydrazine concentrations from 0.01 mM to 1.0 mM) or corresponding amounts of 0.01N HCl alone (control cultures). Fresh medium containing hydrazine was added at 48 hr. After 3 days, the cells were fixed in medium with 2% glutaraldehyde (30 min at room temperature, and overnight at 4°C), and the adherent cells on the glass coverslips were processed for SEM according to the procedures described by Cohen et al. (1968). The coverslips were rinsed 5 min in phosphate buffered saline and run through a series of 5 minute dehydrations in aqueous ethanol (50-100%). This was followed by a series of 10 minute ethanol-freon dehydrations (30-100% freon 113), critical point drying (Omar SPE-900EX) utilizing freon 113, and gold evaporation (Technis Hummer II, 3 min at 10 V). Coverslip specimens were mounted with silver paint on aluminum studs and analyzed on a Hitachi HS500 scanning electron microscope (15-20 KV, tilt angle of 30-55°). Random samples of cells on the coverslips were examined with regard to the quantity of cell surface projections (light, moderate, heavy surface detail) that each cell displayed. Tabulations of the number of cells displaying each type of surface detail were prepared from each specimen by an investigator who was unaware of which specimen corresponded to each hydrazine concentration. Evaluation of the observed data was verified with the student's t-test (confidence level at least 95%).

Heart Culture/Electrophysiology

Neonatal rat (1-2 days old) ventricular cells were cultured in Rose chambers according to the methods previously described (Kitzes and Berns, 1979). Glass micropipette microelectrodes filled with 2.7 M K citrate were utilized for intracellular recording. Electrodes were selected with resistances between 20 megohms and 50 megohms. Individual contracting myocardial cells were selected for impalement by observation through a Nikon inverted phase microscope. The microelectrode was carefully lowered into the selected cell using a de Fonbrune pneumatic micromanipulator, and the electrical properties of the cell were recorded and analyzed according to the procedures described earlier (Kitzes and Berns, 1979). Recordings were made both before exposure to hydrazine and during the indicated times after

the medium bathing the cells was replaced by medium supplemented with 0.01 mM, 0.1 mM or 1.0 mM hydrazine.

RESULTS

Figure 1 demonstrates the basic dose related growth response of four cell types to hydrazine concentrations of 0.01 to 10 mM. Although the different cell types expressed varying degrees of sensitivity towards hydrazine, all cell types showed several common responses. Hydrazine was cytotoxic to all populations tested at dosages ranging from 0.5 mM to 4 mM, depending on the cell type. At lower concentrations, hydrazine produced a dose dependent suppression (but not complete inhibition) of population growth with 0.01 mM to 1.0 mM being the threshold range of response for most cell types tested. For each cell type, there appeared an optimum dose (OD) of hydrazine which initially suppressed population growth from control levels and yet allowed the treated population to recover to log phase growth. This dosage appeared to be 1.0 mM for PTK_2, 1.0 mM for A6, 0.1 mM for W138 and 0.05 mM for CH cells. The return of OD treated populations to log phase growth implies that either the action of hydrazine on a cell may be short-lived or that non-hydrazine sensitive cells in the population are being selected for.

In addition to being dose dependent (Fig. 1), the growth suppression seen in both A6 and PTK_2 cell types appeared to be related to the length of time of hydrazine exposure. The effects caused by various exposure lengths of 1.0 mM hydrazine on PTK_2 and A6 cells are illustrated in Fig. 2. These cultures were replenished with medium containing fresh hydrazine at the initial exposure point (arrow) and at every subsequent data point up to 24 hours or 96 hours. The cultures designated "continous" received fresh hydrazine-containing medium at each data point throughout the experiment. Beyond the designated time of treatment, the medium was replaced at each data point with fresh medium containing no hydrazine. The net population increase observed in cultures receiving fresh hydrazine containing medium at every point beyond induction is noteworthy. It suggests either a selection of genetically resistant cells or a physiological adaptation of cells to hydrazine. It is not likely that the observed effect is due to hydrazine breakdown in the medium; if this were occurring, at least an initial depression in growth rate should be observed after exposure to each fresh hydrazine application.

To test for the selection of hydrazine resistant cells, OD treated A6 cells which achieved log phase growth (day 10) were replated and treated anew with fresh hydrazine (Fig. 3). The "selected" cells responded to hydrazine treatment with severe population growth suppression similar to the untreated "naive" cells. The difference between the growth rates of "selected" and "naive" cells was insignificant when compared to the control growth rate. There was no appreciable difference between growth rates of untreated "naive" and untreated "selected" controls (data not shown). These results indicate that no genetic selection was operating in the observed recovery from hydrazine-induced growth suppression. Further attempts to detect hydrazine-induced genetic mutation in several cell lines via ouabain resistance and growth in sloppy agar were completely unsuccessful.

Besides unsuccessful mutation assays, several other experiments were performed to detect hydrazine-induced cellular anomalies which could account for the behavior of the experimental populations. Examination of earlier Coulter counter data of control and hydrazine treated populations revealed an apparent cell size increase in the experimental cultures (Fig. 4). In a typical dose response growth curve of 72 hr treated populations, the experimental cultures had a measurably larger mean cell volume than the control cultures (plots of cell populations are taken from day 5 samples of control and 1.0 mM treated A6 and PTK_2 cells shown in Fig. 1). Further microscopic analysis of PTK_2 cells revealed significantly more multinucleate cells in 1.0 mM hydrazine treated populations than in control populations (Table 1, Fig. 5). This threefold increase in multinucleation resulted in each of triplicate experiments performed.

The increase in cellular multinucleation suggests that hydrazine may act to produce either abnormal mitosis resulting in multiple nuclei or cell surface alterations promoting cell fusion. Experiments were undertaken to test for both possibilities in 1.0 mM treated cell populations. Because PTK_2 cells remain perfectly flat throughout mitosis (Rattner and Berns, 1974), it was possible to carefully observe mitosis by light microscopy. Studies employing still and time lapse photography revealed no difference in mitotic abnormalities between control and treated populations. However, evidence for increased cell fusion in hydrazine treated populations implicated the cell surface as a possible target site for hydrazine action (Table 2). In a mixed population of PTK_2 cells having either regular or ^3H-tagged nuclei, the presence of multinucleated cells containing both types of nuclei (tagged and untagged) suggested that cell fusion was occurring. There was a fivefold increase of these types of cells in the hydrazine treated populations as compared to the control populations (Fig. 6 and Table 2). Furthermore, the quantity of these cells comprising the entire multinucleate population of a culture was significantly higher (1.5 fold) in the hydrazine exposed cultures than in control cultures.

To further investigate membrane effects, cell surface morphologies of hydrazine treated and control A6 cells were studied (Fig. 7). Table 3 presents data showing the effect of hydrazine on the cell surface. It is clearly evident that there is a significant, inverse relationship between the hydrazine concentration a cell received and the amount of SEM detectable cell surface projections it displayed. Furthermore, this inverse relationship is concentration dependent, paralleling the results of the growth response curves.

Since the cell fusion data and the SEM data implicated the cell membrane as a primary site of hydrazine action, additional studies were undertaken to examine the effect of hydrazine (0.01 mM to 1.0 mM) upon membrane associated electrical and contractile activities of neonatal rat myocardial ventricular cells in culture.

A typical intracellular recording of spontaneous action potentials recorded in a rhythmically contracting heart cell is shown in Fig. 8a. The resting membrane potential is approximately -60 mV, and the action potentials occur at a rate of approximately 1 per second. In Figs. 8b and 8c are shown typical recordings 10 min and 20 min following exposure of the culture to 0.01 mM hydrazine. Three effects are apparent: (1) a depolarization of the resting membrane potential, (2) a reduction of action potential amplitude, and (3) a disruption of discharge rhythmicity. These electrical changes were accompanied by a disruption of the rhythmic contractile behavior of the cell. However, electrical activity returned to almost normal status 30 min after cells initially received medium containing 0.01 mM hydrazine (Fig. 8d).

Exposure to 0.1 mM hydrazine (Fig. 9b) resulted in (1) a more severe reduction in membrane potential that is still evident 15 min after exposure, (2) a complete absence of action potential discharge, and (3) arrhythmic baseline activity. During this time, the cell was not contracting. At this hydrazine concentration, the cells resumed normal electrical and contractile activity after 45 min in the hydrazine supplemented medium (Fig. 9c). Cells exposed to 1.0 mM hydrazine (Fig. 10) did not recover after 45 min to 1 hr in the experimental medium. At this time (Fig. 10), only very aberrant electrical activity accompanied by occasional small and typical contractile activity was observed. However, these cells resumed apparently normal electrical and contractile activity 15 min after the hydrazine-containing medium was replaced with normal medium (data not shown).

DISCUSSION

The purpose of this study was to examine the basic cellular effects of hydrazine. Previous studies have linked hydrazine and its derivatives with carcinogenic and mutagenic effects. Carcinogenic effects of the hydrazine analogue 1, 2, dimethyl hydrazine have been reported in vivo in studies of rodent intestines (Barlka and Tutton, 1977, 1978; Jacobs, 1977; Mak and Chong, 1978; Richards, 1977; Sunter et al. 1978; Toth et al. 1976) and blood vessels (Toth and Wilson, 1971). Hydrazine has been shown to mutate DNA from a variety of sources (Raphalian, 1966; Dave, 1977; Brown, 1967; Brown et al., 1966; Gupta and Grover, 1970; Kak and Kaul, 1975; Kimball, 1977; Kimball and Hirsch, 1975, 1976; Lemontt, 1977). These effects appear as the result of the chemical treatment of selected target tissues under specific conditions. In this study, however, a diverse variety of tissue culture cell types were employed to ascertain the basic cellular effects of hydrazine.

The noted hydrazine-induced growth effects shown in this study could be classified into two basic types of responses. First of all, the cells demonstrated a concentration dependent response to hydrazine treatment (Figs. 1, 8, 9, 10 and Table 3). In these studies, different hydrazine concentrations, usually spanning 3 or 4 orders of magnitude, caused effects ranging from imperceptible cellular changes to lethality. Within this concentration range, the cells could survive, proliferate, and function. Secondly, in the growth response curves, the growth rate remained depressed while cells received intermittent rechallenges to the higher hydrazine concentrations; however, the growth rates resumed normal log phase levels when hydrazine containing medium bathing the cells was replaced with normal medium (Figs. 2, 3 Control--see Methods). At the lower concentrations of hydrazine treatment, the cells exhibited an initial depression in growth, but, upon re-exposure to fresh hydrazine, they appeared to attain a normal growth rate. Likewise in heart cell cultures, the aberrant electrical and contractile effects produced by the presence of 1.0 mM hydrazine abated when these cells were washed and bathed in normal medium. In the presence of low hydrazine concentrations, treated heart cells appeared to show some recovery from the observed effects (Figs. 8, 9).

The mechanism whereby treated cells rebound in the presence of fresh hydrazine is unclear. It seems possible that this "tolerance" or "recovery" may be due to an increased capacity of exposed cells to inactivate hydrazine or its effectual metabolite. The production of "tolerant" cells in the presence of hydrazine could also suggest the selection of genetically resistant cells or the alteration of an affected organelle to a hydrazine-refractory state. Regardless of the mechanism, it appears that hydrazine evokes some form of selection or tolerance. This observation is supported by data showing that entire populations, rather than a few selected cells, seem altered by hydrazine treatment (Figs. 3, 4). Furthermore, Fig. 3 indicates that, in the tissues studied, hydrazine acted in a disruptive but non-mutational manner. It therefore seems logical that hydrazine may elicit these concentration dependent and reversible responses by actively and non-mutagenically interfering with a common cellular site in a wide spectrum of cell types. The SEM and electrophysiological studies, which were done on very different cell types, suggest that the cell surface may be a common target site of hydrazine action. Furthermore, the increase of multinucleation by cell fusion seen in hydrazine treated cultures also indicates that the cell surface is a major site of this compound's action.

The above observation is further supported by the literature in which hydrazine derivatives have been shown to elicit a wide variety of effects in studies performed on biological membranes (Balduini, et al., 1977; Barkla and Tutton, 1977; Braun and Wolfe, 1977; Caroni, 1977; Jain and Subrahamanyam, 1978; Jain et al., 1977; Jain et al., 1978; Katsumata et al., 1977; McLean et al., 1978; Tsau et al., 1977; Walter et al., 1978; Zimmer, 1977). The observations of these numerous studies support the view that the hydrazine-induced cellular

effects seen in our data stem from membrane interactions with this compound. Furthermore, it seems logical that such a strong reducing agent as hydrazine would directly affect the cell structure it first contacts.

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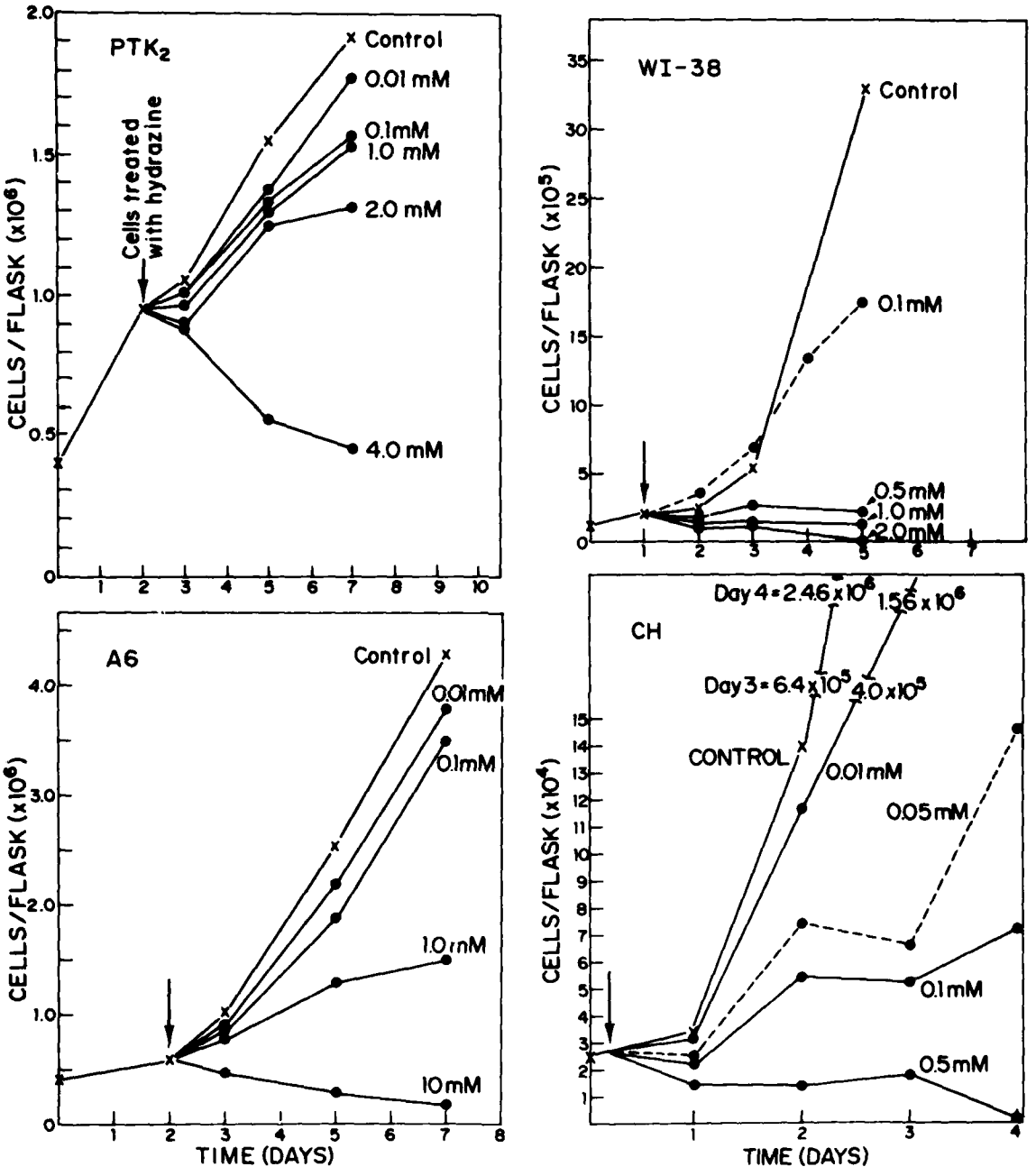
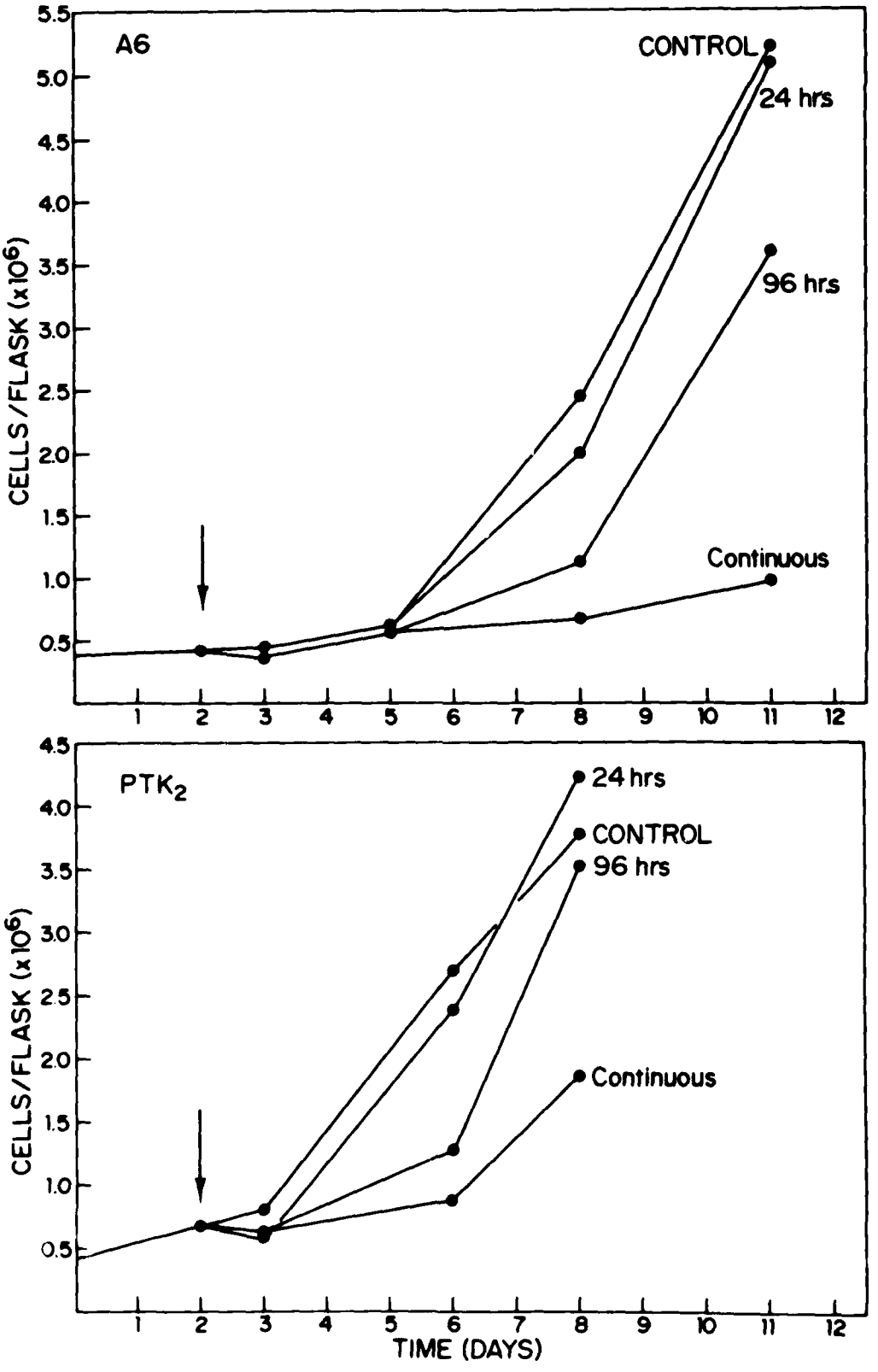


Fig. 1. Growth response curves of four cell lines (PTK_2, WI-38, A-6 and CH to solutions containing the indicated hydrazine concentrations. Cells were exposed to fresh hydrazine at every point along the graph throughout the duration of the experiment. Arrows indicate the point of initial treatment.

Fig. 2


A6
CONTROL
24 hrs
96 hrs
Continuous
CELLS/FLASK (x10⁶)
PTK2
24 hrs
CONTROL
96 hrs
Continuous
TIME (DAYS)

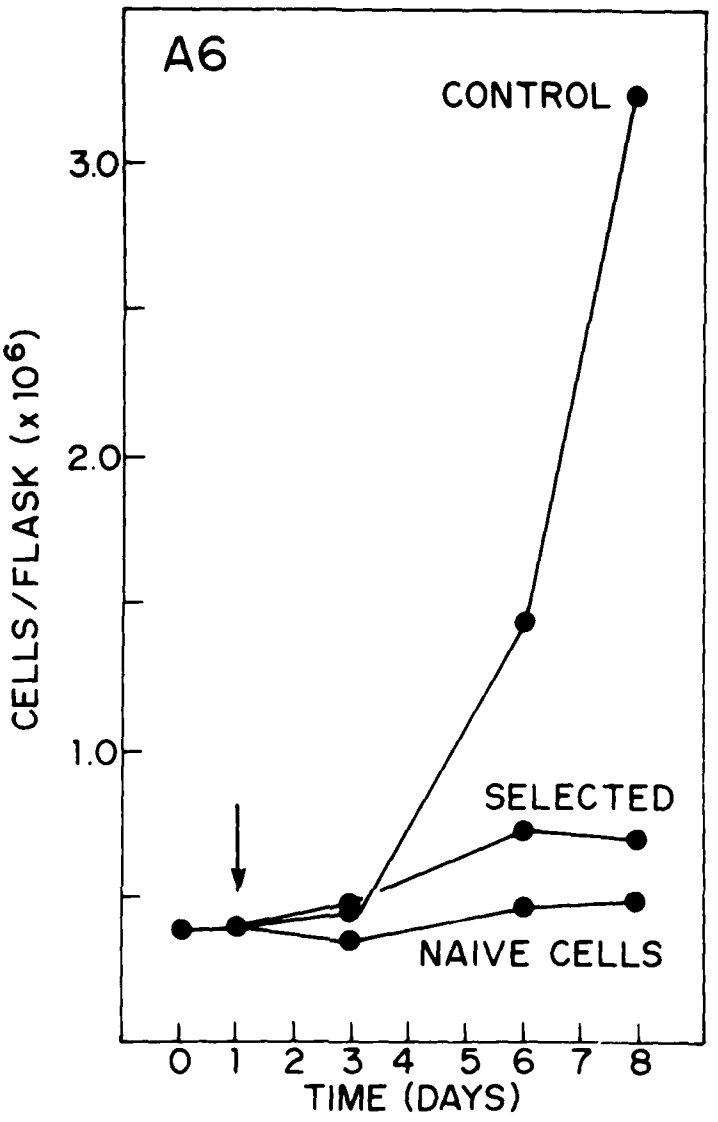


Fig. 3. Effects of 1.0 mM hydrazine on the growth rates of two sub-populations of A6 cells. "selected" cells, which had been grown to confluency in the presence of 1.0 mM hydrazine, and "naive" or unselected A6 cells. Both populations were treated with hydrazine throughout the experiment from initial time of exposure (indicated by arrow).

Fig. 2. (see preceding page) Growth curves of A6 cells and PTK_2 cells treated with 1.0 mM hydrazine for varying exposure periods. Established cultures received medium containing fresh hydrazine at each point from the initial point of treatment (indicated by arrow) up to the designated time - 24 hours, 96 hours, or throughout the duration of the experiment (continuous). Beyond the indicated treatment, the cultures received fresh medium without hydrazine at each subsequent point.

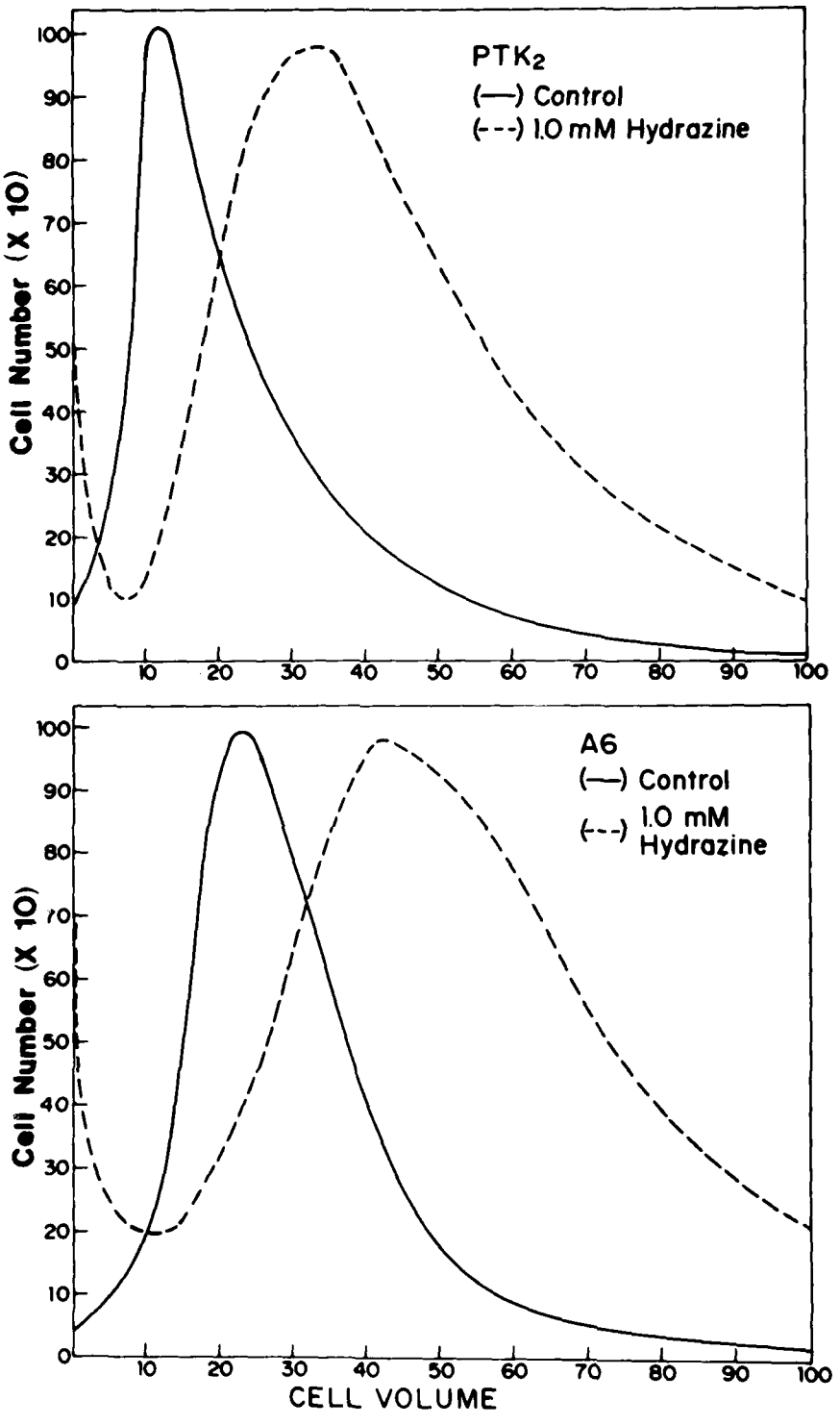


Fig. 4. Effect of 1.0 mM hydrazine on the relative distribution of individual cell volumes from PTK_2 and A6 suspended cell cultures (from Fig 1, day 5) as determined by Coulter counter channelyzer plots. Abscissa represents 100 graded relative cell volume channels. Ordinate illustrates the number of cells in a population fitting a certain channel size; maximum cell number per channel = 1,000.

Table 1. Incidence of multinucleation occurring in both control PTK_2 cultures and experimental cultures treated for 2 days with 1.0 mM hydrazine.

	Uninucleated cells (%)	Multinucleated cells (%)	Total # cells
Control Cultures	92	8	170
Experimental Cultures (hydrazine treated)	78	22*	159

* Hydrazine treated cultures had significantly more multinucleate cells than did controls (at least 95% confidence).

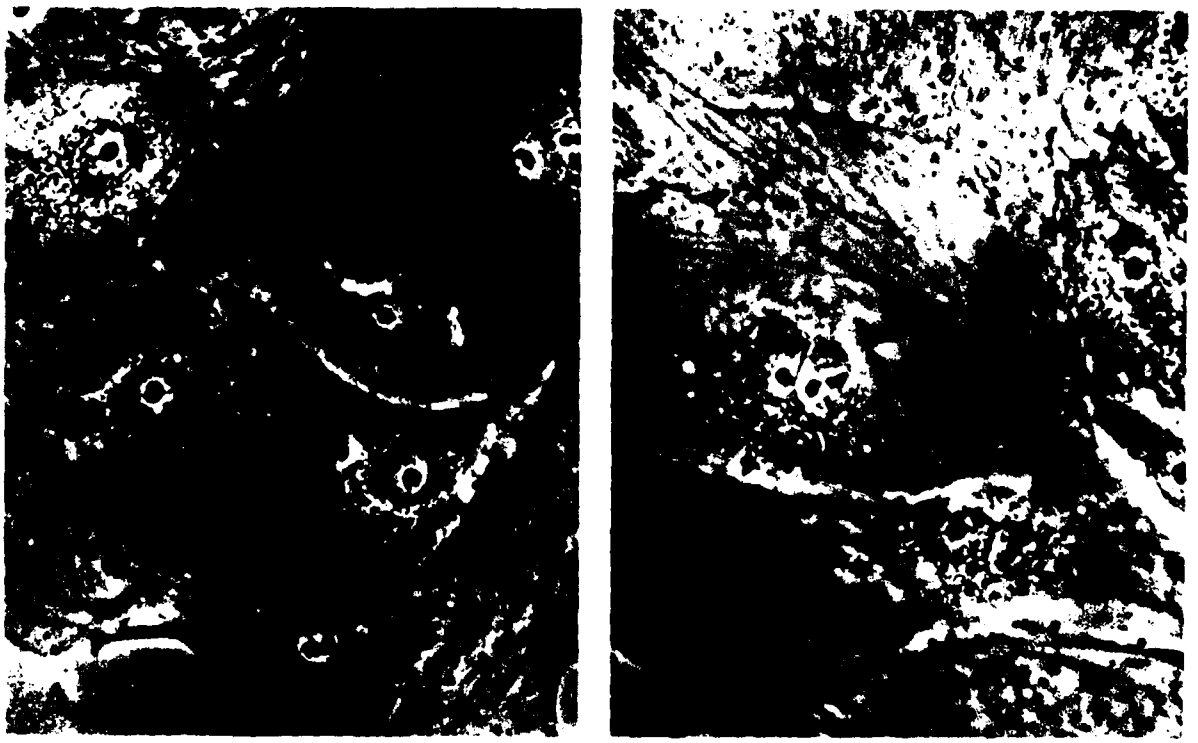


Fig. 5. Phase contrast micrographs of PTK_2 control cultures (A) and 72 hr, 1.0 mM hydrazine treated cultures (B). Magnification = 2200X.

Table 2. Incidence of cell fusion occurring within the multinucleated populations in both control PTK_2 cultures and experimental cultures treated for 3 days with 1.0 mM hydrazine.

	Uni nucleated cells (%)	Multi-nucleated cells (%)	Similarly[a] Labeled Nuclei (%)	Dissimilarly[b] Labeled Nuclei (%)
Control Cultures	95	5	4	1
Experimental Cultures	85	15	10	5*

[a] Refers to multinucleated cells containing either all normal or all ^{3}H-thymidine tagged nuclei.

[b] Refers to multinucleated cells containing both normal and ^{3}H-thymidine tagged nuclei.

* Hydrazine treated cultures had significantly more dissimilarly labeled multinucleated cells than controls did (confidence level at least 95%).

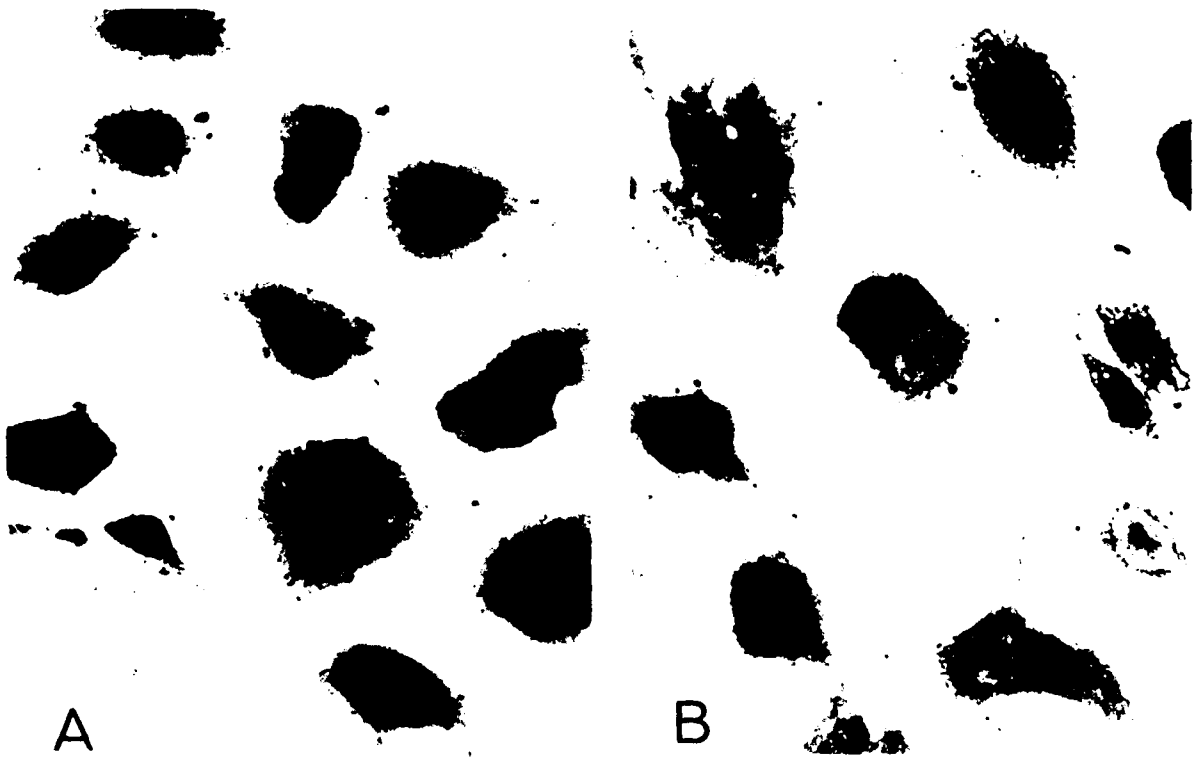


Fig. 6. PTK_2 cell cultures, stained after autoradiography with methylene blue, from control (A) and 32 hr, 1.0 mM hydrazine treated (B) cultures. Cells containing both labeled and unlabeled nuclei were formed by cell fusion. Magnification = 1900X.

Table 3. Effects of various hydrazine dosages on SEM detectable Xenopus cell surface projection.

Hydrazine Concentration (mM)	Cells with Light Surface Projection (%)	Cells with Moderate Surface Projection (%)	Cells with Heavy Surface Projection (%)	Total # Cells Sampled
0	24	37	39	196
0.01	35.5	31.5	33	186
0.1	60	31.5	8.5	188
1.0	79	17	4	203

* Statistical analysis verified a significant difference in the quantity of light or heavy cell surface projection present between populations of control cultures and populations receiving either 0.1 mM or 1.0 mM hydrazine (confidence level at least 95%).

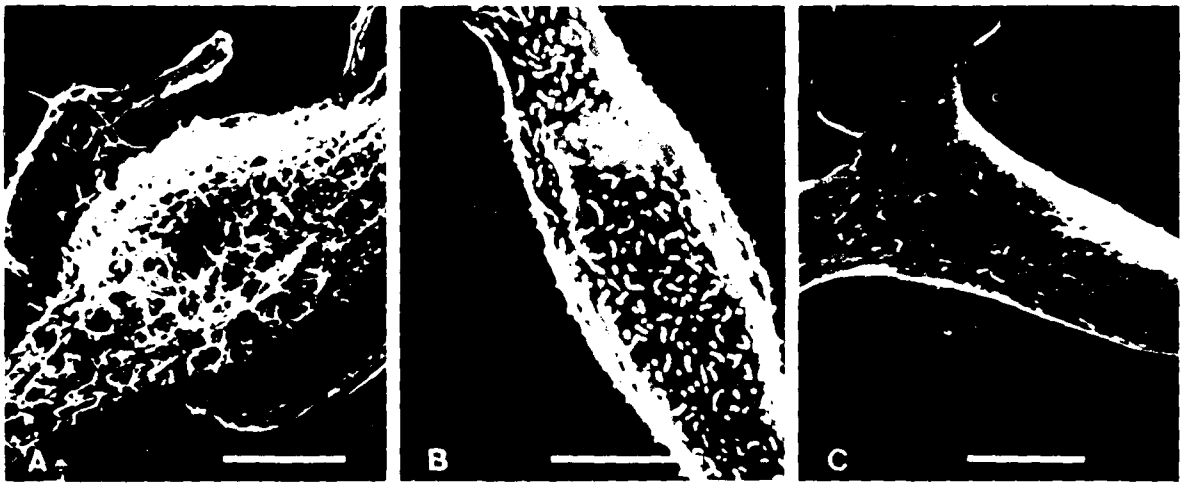


Fig. 7. Scanning electron micrographs of A6 cells which typify (A) heavy, (B) moderate, and (C) light cell surface projections. Bar = 5um.

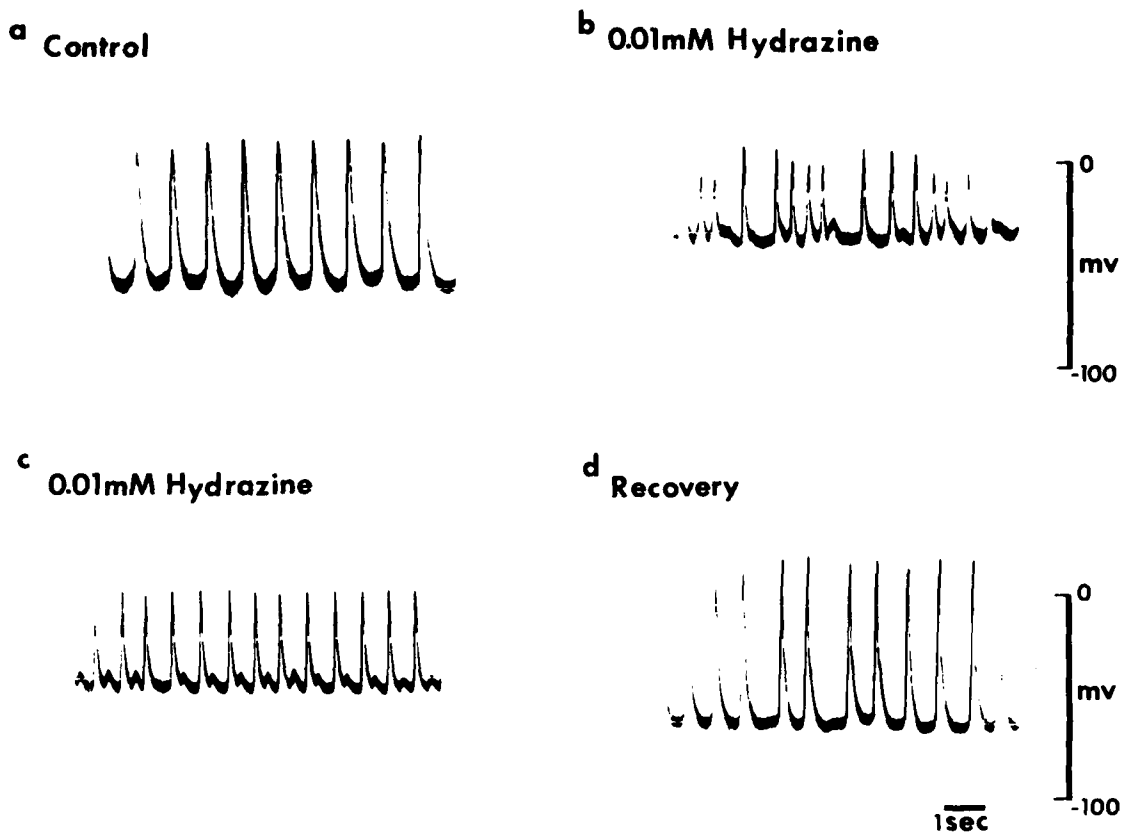


Fig. 8. Effects of exposure to 0.01 mM hydrazine on the spontaneous intracellular electrical activity of rhythmically contracting myocardial cells in culture: (A) control activity (no exposure to hydrazine); electrical activity after exposure to 0.01 mM hydrazine for (B) 10 minutes; (C) 20 minutes; and (D) 30 minutes.

Fig. 9

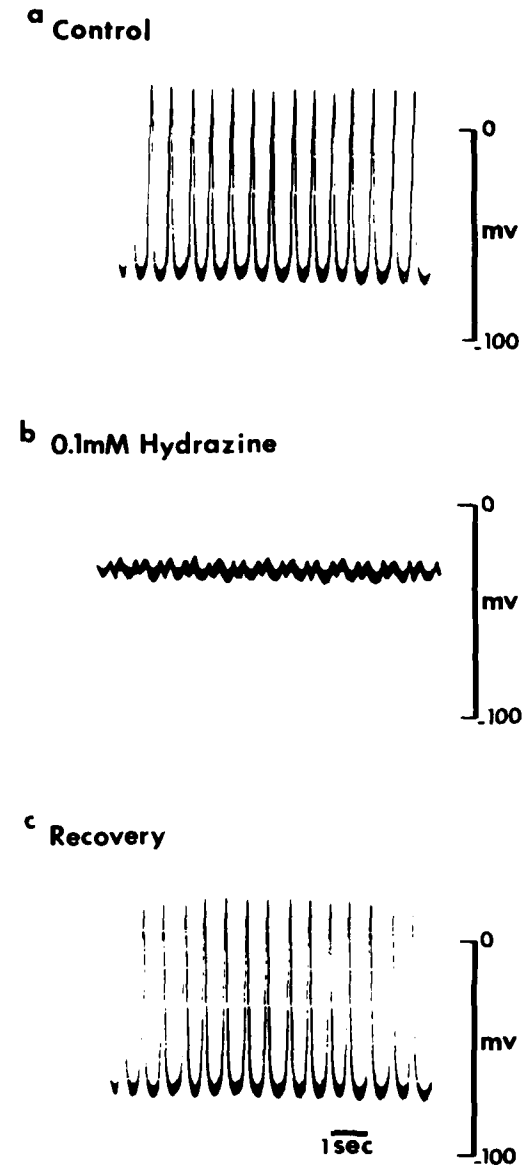


Fig. 10

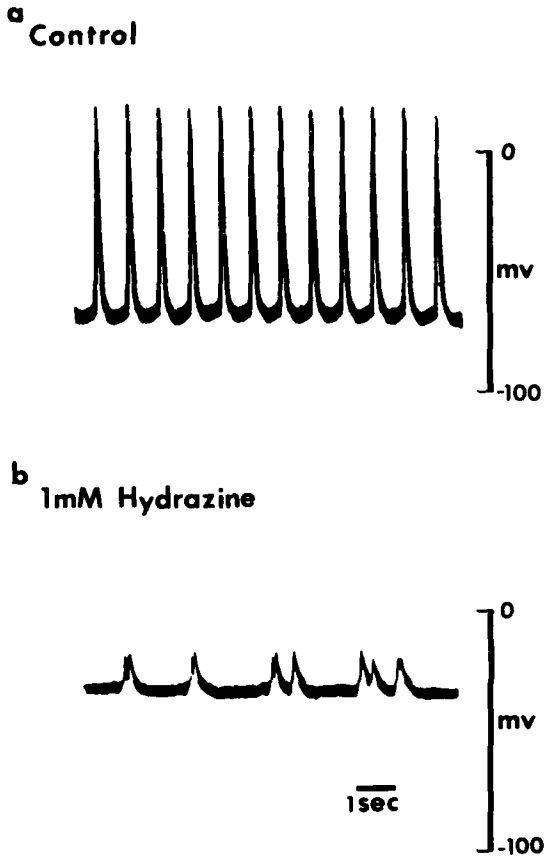


Fig. 9. Effects of exposure to 0.1 mM hydrazine (A) Control; (B) 15 minutes after culture received 0.1 mM hydrazine solution; (C) 45 minutes after culture received 0.1 mM solution.

Fig. 10. Effects of exposure to 1.0 mM hydrazine (A) control activity, (B) aberrant electrical activity after 1 hr in 1.0 mM solution hydrazine.

THE ONCOGENIC HAZARD FROM CHRONIC INHALATION OF HYDRAZINE

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SUMMARY

Hydrazine is used in the United States Department of Defense as a bipropellant mixture with unsymmetrical dimethylhydrazine in missile operations, as a monopropellant to power aircraft emergency power systems, and as an oxygen scavenger in shipboard boiler water treatment. It also finds a wide variety of uses in the civilian community. For more than a decade it has been known that hydrazine administered orally at high doses in mice caused increased tumor formation. However, no data were available to evaluate the cancer-producing hazard from occupational inhalation exposures. Studies were therefore conducted to evaluate the long-term effects of airborne hydrazine at levels near the present and proposed Threshold Limit Value concentrations. Repeated daily inhalation exposure to 5 parts per million (ppm) hydrazine induced nasal tumors in Fischer 344 male and female rats and in male Golden Syrian hamsters. Repeated exposure to 1 ppm also produced nasal turbinate tumors in rats and pulmonary adenomas in female C57B1/6 mice. The inhalation exposures to the rodents were conducted for 6 hours per day, 5 days per week over a 12-month period. The hamsters were held for an additional 12-month postexposure observation period and the survivors were necropsied. Rats and mice were held 18 months postexposure. The nasal turbinate tumor incidence in rats was dose related. Increased tumor incidence occurred in both mice and hamsters at the maximum tolerated repeated inhalation dose. No statistically significant tumorigenic effects occurred after repeated exposure to 0.05 and 0.25 ppm hydrazine concentrations which spanned the American Conference of Governmental Industrial Hygienists recommended Threshold Limit Value.

BACKGROUND

Hydrazine (N_2H_4) is a highly reactive reducing agent which is widely used as an intermediate in organic synthesis and either singly or in combination with other hydrazines as a missile propellant. An important and increasing use of hydrazine is that of a boiler feed water additive as an oxygen scavenger. It is a colorless polar liquid, weakly basic, and it fumes in air. It has a slightly ammoniacal odor.

Clark[1] provided a detailed review of the toxicology and pharmacology of propellant hydrazines. Hydrazine is a strong convulsant at high doses but may cause central nervous system depression at lower doses. Animals may die acutely of convulsions, respiratory arrest, or cardiovascular collapse within a few hours of an acute exposure by any route of administration, or may die two to four days later of liver and kidney toxicity.[2,3] Jacobson et al.[4] reported the 4-hour LC_{50} value as 252 ppm (330 mg/m^3) for the mouse and 570 ppm (750 mg/m^3) for the rat. House[5] exposed monkeys, rats, and mice to a hydrazine concentration of 1.0 ppm continuously for 90 days. Though mortality was very high, some animals survived. Ninety-six percent of the rats and 98% of the mice died during the exposure, while monkeys proved to be the most resistant species with only a 20% mortality. Comstock et al.[6] exposed dogs, in separate experiments, to 5 and 14 ppm. Two dogs survived the repeated six-hour exposures to 5 ppm hydrazine for six months, and two of four dogs lived after 194 six-hour exposures to 14 ppm. Two of four dogs died during the third and fifteenth weeks in a debilitated condition. The dog that died during the fifteenth week had a severe convulsive seizure prior to death. Prior to death, both dogs showed signs of anorexia and general fatigue. Changing diets and forced feedings resulted in the survival of the remaining two dogs.

A six-month chronic inhalation study of hydrazine was reported by Haun and Kinkead[7] which employed four exposure groups and an unexposed control group. Each group was comprised of 8 male beagle dogs, 4 female rhesus monkeys, 50 male Sprague-Dawley rats, and 40 female ICR mice. The experimental groups were exposed to vapors of hydrazine either at concentrations of 1.0 or 0.2 ppm continuously, or at 5.0 and 1.0 ppm intermittently. The continuous exposures were designed to approximate the same weekly doses of hydrazine received by the intermittent exposure groups, with continuously exposed animals receiving 168 and 33.6 ppm-hours of hydrazine/week and intermittently exposed animals 150 and 30 ppm-hours/week. Dogs exposed at the higher dose levels, either intermittently or continuously, exhibited 10-20% reductions in erythrocyte, hematocrit, and hemoglobin values which continued throughout the six-month exposure but returned to

control values within two weeks after the exposure ended. Hematology values for dogs exposed to lower doses remained within the normal limits of the control group.

Rats showed a dose-related growth rate depression and a sustained difference in group average weights of up to 35 grams throughout the exposure. Weight loss in dogs which occurred only in the high dose group was recovered within two weeks postexposure, suggesting that the loss was due to appetite suppression. Gross and microscopic examination of tissues from these animals taken at termination of the exposure showed fatty liver changes in mice and dogs at the high exposure dose levels but no exposure-related changes in the livers of monkeys and rats.

Ten mice and 10 rats from each of the exposure groups were held for a year post-exposure period. Most of the rats in the two high dose groups died within 6-8 weeks postexposure from chronic pulmonary disease. This infection spread to the other groups housed in the same animal room. Consequently, none of the rats survived long enough to evaluate the carcinogenic potential of inhaled hydrazine for this species.

Approximately half of the mice in each group were alive one year postexposure. Tumorigenic changes in these mice were reported by MacEwen et al. in 1974.[8] Mice exposed to the high doses (continuous exposure to 1 ppm hydrazine or intermittent exposure to 5.0 ppm) had increased incidences of alveolargenic carcinomas, lymphosarcomas, and hepatomas. Both lower dose groups had an increased incidence of alveolargenic carcinomas when compared with unexposed controls. The total tumor incidence appeared to be dose related: approximately 87% tumor incidence occurred at the high dose level; 33% at the low dose level; and 12% in the unexposed control group. Although the group sizes were very small, the findings were important in that they demonstrated tumorigenic response at the current Threshold Limit Value.

Since hydrazine inhalation at the Threshold Limit Value increased the incidence of pulmonary tumors in mice, a more comprehensive oncogenic study of hydrazine effects on multiple species was undertaken.

EXPERIMENTAL DESIGN AND RESULTS

The objectives of this study were to evaluate (a) the chronic effects of inhaled hydrazine on rats, mice, hamsters, and dogs and (b) the oncogenic potential of hydrazine in rodents observed for a maximum period of 1-1/2 years after one year of industrial-type inhalation exposure. The animals used in this study were C57B1/6 mice obtained from the Jackson Laboratories, CDF (Fischer 344 derived) albino rats from Charles River, Engle Golden Syrian hamsters, and beagle dogs. The number of animals of each species and sex are listed in Table 1 which also shows the exposure concentrations.

TABLE 1. EXPERIMENTAL DESIGN FOR HYDRAZINE INHALATION EXPOSURE CONCENTRATIONS

Hydrazine Concentration, ppm	Species and Sex	Number of Animals
0.05	Rats, male	100
	Rats, female	100
	Mice, female	400
0.25	Hamsters, male	200
	Mice, female	400
	Rats, male	100
	Rats, female	100
	Dogs, male	4
	Dogs, female	4
1.0	Hamsters, male	200
	Mice, female	400
	Rats, male	100
	Rats, female	100
	Dogs, male	4
	Dogs, female	4
5.0	Rats, male	100
	Rats, female	100
	Hamsters, male	200
Control	Rats, male	150
	Rats, female	150
	Mice, female	800
	Hamsters, male	200
	Dogs, male	4
	Dogs, female	4

The exposure concentrations were selected to span the range from a certainly toxic level to the current Occupational Safety and Health Administration (OSHA) Threshold Limit Value for exposure to hydrazine (1 ppm) and the proposed American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value of 0.1 ppm. The 5 ppm exposure concentration was selected as a maximum tolerable exposure dose which would

produce some biological response without causing death in hamsters and rats. Mice and dogs were not exposed at this concentration, because prior studies (Haun and Kinkead[7]) had shown that repeated daily exposures to 5 ppm hydrazine caused death in these species.

The inhalation exposures were conducted on a 6 hour/day, 5 day/week schedule for a one-year period without exposures on weekends and holidays. The animals were exposed in Thomas Dome exposure chambers (Thomas[9]) at a slightly negative pressure (725 mm Hg) to insure a complete seal and to prevent contamination of the surrounding laboratories and personnel. All animals were observed hourly during the 12-month hydrazine exposure phase of the study and daily during the postexposure phase. Rats, dogs, and hamsters were weighed individually at biweekly intervals during exposure and monthly during the postexposure period. Mice were weighed in cage groups and group means followed on a monthly schedule throughout the entire study.

Blood samples were drawn from dogs at biweekly intervals during the exposure phase and clinical determinations made for the following battery of tests:

RBC	Glucose
WBC	Total Protein
HCT	Albumin
HGB	Globulin
Sodium	A/G Ratio
Potassium	SGPT
Calcium	Alkaline Phosphatase

Animals that died or were killed during the study were necropsied following the National Cancer Institute protocol. The necropsy consisted of an external examination, including all body orifices, and the examination and fixation of portions of approximately 44 tissues.

Although not dose dependent, growth was reduced in all hydrazine-exposed rats during exposure, but the effect was most significant in the male rats exposed to the 5 ppm concentration. The differences between exposed and control animals were maintained at relatively constant levels during the first 12 months postexposure but became less significant during months 25 to 30 of the study as the weight decline of the aging animals was observed. The effect of depressed growth in female rats was not as pronounced as in males during the exposure phase but was significant and became more noticeable during the postexposure observation period. Hamster body weights were depressed for all exposure groups but also exhibited an inexplicable cyclic phenomenon common to all groups exposed as well as the unexposed that was relatively severe in all groups. In the final months, only the 5 ppm hydrazine exposed group continued to show a significant weight difference from controls. Mice were not exposed to the 5 ppm hydrazine atmosphere. Body weights of mice were unaffected by chronic exposure to inhaled hydrazine at 1 ppm or less.

There was no significant increase in the mortality experience of the hydrazine-exposed mice, rats, hamsters, or dogs. Gross histopathologic examinations were performed on all rodents that died during the course of the study or were sacrificed at completion of the postexposure period. Histopathologic examinations were conducted in accordance with the National Cancer Institute protocols on approximately 33 tissues from all animals with the exception of a few in which postmortem changes were extensive or cannibalism prevented examinations.

Surviving hamsters were sacrificed one-year postexposure, and their tissues were examined by pathologists of the Veterinary Science Division at Brooks Air Force Base, Texas. Tumor and nontumor nomenclature was developed by this group for automated data processing of the results from hamsters. Tumor incidence tables were compiled, and statistical analyses, using the Fisher Exact Test, were performed by the University of California, Irvine, staff. Since rat mortality was very low after one-year postexposure, 10% of the survivors were sacrificed and tissues collected as previously described. The study was terminated after 30 months (18 months postexposure), and all surviving rats were necropsied. Mouse mortality approached 90% in the 18th postexposure month for the first set of animals including the 0.05 ppm and 0.25 ppm hydrazine-exposed mice and their controls. The second set of mice, including the 1 ppm hydrazine exposure group and their controls, was terminated at 132 weeks which was 3 weeks longer than the first set. Tissues from both rats and mice were sent to the Huntingdon Research Centre in Huntingdon, England, for histopathologic examination. Rats were examined by Dr. C.P. Cherry and mice by Dr. J.M. Offer under the supervision of Dr. D.E. Prentice.

Table 2 shows the tumor incidence in the various groups of exposed and control hamsters. The outstanding finding in hamsters is a statistically significant increase in benign nasal polyps. These tumors were seen in 16/160 of the 5 ppm exposed animals; only one in the control group. The only other tumor types of possible importance are those of the colon in the 5 ppm exposure group. There were three primary adenocarcinomas, one benign leiomyoma, and one benign papilloma. When these tumor types were separately subjected to the Fisher Exact Test, none showed statistical significance. There was a rather large incidence of cortical adenomas in the adrenals of all groups of exposed hamsters but with incidence rates lower than that in the control group. This type of tumor is commonly seen in aged hamsters. Incidence of other tumors in the various organs was low. No biological significance is attached to the increase in benign thyroid

adenomas limited to the 0.25 ppm hydrazine exposure group. The reduced incidence of adrenal cortical adenomas may indicate some antineoplastic activity as will also be seen with leukemia incidence in rats.

TABLE 2. TUMOR INCIDENCE IN CONTROL AND HYDRAZINE-EXPOSED MALE GOLDEN SYRIAN HAMSTERS†

TUMOR TYPE	Unexposed Controls	0.25 ppm Exposed	1.0 ppm Exposed	5.0 ppm Exposed
Nares, Trachea, Bronchi				
Polyp (B)	1/181	0/154	1/148	16/160**
Basal Cell (P)	0/181	0/154	1/148	0/160
Basal Cell (B)	0/181	0/154	0/148	1/160
Adenoma (P)	0/181	1/154	0/148	0/160
Adenoma (B)	0/181	0/154	0/148	2/160
Lung				
Bronchogenic Adenoma (P)	1/179	0/154	1/146	0/155
Bronchogenic Adenoma (B)	0/179	0/154	0/146	2/155
Liver				
Reticulo-endotheliomas (B)	1/180	0/160	0/148	0/159
Spleen				
Hemangioma (P)	1/160	1/129	0/130	2/138
Reticulo-endotheliomas (P)	1/160	2/129	0/129	0/138
Reticulo-endotheliomas (B)	1/160	0/129	0/129	0/138
Bone Marrow, Blood				
Myelogenous (P)	0/157	0/134	1/136	0/135
Bone				
Osteoma (P)	0/177	0/152	0/148	1/156
Lymph Nodes				
Reticulo-endotheliomas (P)	5/167	4/143	5/140	6/146
Reticulo-endotheliomas (B)	0/167	1/143	0/140	0/146
Kidney				
Renal Adenoma (P)	1/179	2/164	0/145	0/160
Reticulo-endotheliomas (B)	1/179	0/164	0/145	0/160
Thyroid				
Adenoma (P)	1/145	1/117	0/127	0/137
Adenoma (B)	0/145	4/117*	1/127	0/137
"C" Cell Adenoma (P)	0/145	1/117	0/127	0/137
"C" Cell Adenoma (B)	0/145	0/117	0/127	4/137
Parathyroid				
Adenoma (B)	3/111	3/88	2/82	2/100
Adrenal				
Cortical Adenoma (B)	40/177	18/155	19/141	23/153
Cortical Adenoma (P)	6/177	5/155	3/141	4/153
Stomach				
Papilloma (B)	0/169	1/149	0/140	0/145
Basal Cell (P)	0/169	0/149	2/140	1/145
Pleura, Peritoneum Mesenteries				
Fibroma (P)	0/161	2/152	0/139	0/147
Pancreas				
Islet Cell Adenoma (B)	0/114	0/98	0/99	0/107
Small Intestine				
Adenocarcinoma (P)	1/148	1/140	0/132	0/141

TABLE 2. (CONTINUED)

TUMOR TYPE	Unexposed Controls	0.25 ppm Exposed	1.0 ppm Exposed	5.0 ppm Exposed
Colon				
Adenocarcinoma (P)	0/158	0/146	2/129	3/139
Leiomyoma (B)	0/158	0/146	0/129	1/139
Papilloma (B)	0/158	0/146	0/129	1/139
Total Tumors	0/158	0/146	2/129	5/139**
Skin				
Leiomyoma (B)	0/170	1/161	0/146	0/147
Squamous Cell Carcinoma (P)	0/170	1/161	0/146	0/147
Trichoepithelioma (B)	0/170	1/161	0/146	0/147
Hemangioma (B)	0/170	0/161	1/146	0/147
Fibroma (B)	0/170	0/161	0/146	1/147
Pituitary				
Adenoma (B)	0/163	1/133	0/129	1/138

† - Metastatic tumors in various organs were not counted.
(P) - Primary malignant tumors.
(B) - Benign tumors.
* - Significant at the 0.05 level as determined using the Fisher Exact Test.
** - Significant at the 0.01 level as determined using the Fisher Exact Test.

The nonneoplastic histopathology finding for exposed hamsters included descriptions and discussion of many lesions which occasionally occurred more frequently than in control animals. These probably reflected the aging process or the existence of chronic disease states to which hamsters are susceptible. Analysis of the incidence of such lesions would not elucidate the effect of hydrazine exposure on target organs. Therefore, the data were examined to select specific organ lesions which might have been related to exposure. This examination revealed that lesions in the nares, trachea, and bronchi (considered as one organ in the accounting), lung, liver, spleen, lymph nodes, kidney, thyroid, adrenal, colon, and testes occurred more frequently in exposed animals and could be possible sites of toxic action by hydrazine.

Two important observations emerged:

1. Degenerative disease, characterized by amyloidosis in the livers, spleens, kidneys, thyroids, adrenals; and liver hemosiderosis, kidney mineralization, general degeneration of the adrenals; and senile atrophy, aspermatogenesis, and hypospermatogenesis, is a common finding in all groups of hamsters.

2. The important fact is that these lesions occur with statistically significantly higher frequency in the exposed group; and in most cases, a dose-response relationship can be seen. The implication is that the stress of 12 months of hydrazine exposure at the various dose levels tended to increase the degenerative process in a dose-dependent manner.

Nasal epithelial tumors were observed only in hydrazine-exposed rats. The majority of the epithelial neoplasms were benign and were mainly classified as adenomatous nasal polyps. Small numbers of villous nasal polyps, muco-epidermoid papillomas, and squamous cell papillomas were also noted. The incidence of these benign and several malignant epithelial tumors (shown in Tables 3 and 4) was elevated significantly in the 5 ppm hydrazine-exposed rats of both sexes. An apparent dose-response was noted in that the incidence and degree of significance of the benign tumors were less in the 1 ppm hydrazine exposure groups (only one malignancy was found in both sexes). No tumors of this type were seen in either control group of rats, and only one malignancy of the six tumors was seen in about 400 rats exposed to 0.05 and 0.25 ppm. Most of these tumors were seen after two years with the earliest occurring in a male rat at 88 weeks (36 weeks postexposure) and in a female rat at 98 weeks.

Varying degrees of acute inflammation were observed in the nasal cavity, larynx and/or trachea in some rats from the control and all treated groups. The incidence and severity of the inflammatory changes were greatest in male and female rats from the group receiving 5.0 ppm, and in some of these affected animals, they were associated with focal hyperplasia and/or squamous metaplasia of the epithelium of the nasal cavity, larynx, and trachea. These histopathologic changes were observed in rats dying during the study as well as in the animals killed at the 2-year interim sacrifice and at the 2-1/2-year terminal sacrifice.

TABLE 3. SELECTED TUMORS FOUND IN FEMALE FISCHER 344 RATS AFTER INHALATION EXPOSURE TO HYDRAZINE

TUMOR TYPE	Unexposed Controls (N = 147)	Exposed 0.05 ppm (N = 99)	Exposed 0.25 ppm (N = 100)	Exposed 1.0 ppm (N = 97)	Exposed 5.0 ppm (N = 98)
Nasal cavity:					
Epithelial (Benign)	0 (0)	1 (1)	0 (0)	4 (4)*	31 (32)**
Epithelial (Malignant)	0 (0)	0 (0)	0 (0)	0 (0)	5 (5)**
Pituitary:					
Adenoma	59 (40)	28 (28)	35 (35)	33 (34)	40 (41)
Adenocarcinoma	9 (6)	6 (6)	2 (2)	6 (6)	6 (6)
Thyroid:					
Adenoma	9 (6)	2 (2)	4 (4)	7 (7)	7 (7)
Carcinoma	17 (12)	1 (1)	8 (8)	15 (15)	5 (5)
Adrenals:					
Pheochromocytoma	10 (7)	3 (3)	6 (6)	9 (9)	12 (12)
Uterus:					
Adenoma	1 (0)	0 (0)	0 (0)	2 (2)	3 (3)
Adenocarcinoma	10 (7)	4 (4)	5 (5)	7 (7)	6 (6)
Endometrial stromal sarcoma	0 (0)	2 (2)	1 (1)	1 (1)	3 (3)
Lymphoreticular Tissue:					
Leukemias	41 (28)**	18 (18)	21 (21)	13 (13)	13 (13)
Sarcomas	4 (3)	4 (4)	4 (4)	2 (2)	6 (6)
Mammary gland:					
Adenoma	4 (3)	4 (4)	6 (6)	8 (8)	8 (8)
Fibroadenoma	28 (19)	20 (20)	11 (11)	18 (19)	19 (19)
Adenocarcinoma	2 (1)	1 (1)	2 (2)	2 (2)	3 (3)
Liver:					
Liver cell tumor	3 (2)	0 (0)	0 (0)	6 (6)	3 (3)
Lung:					
Bronchial adenoma	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)

*Significant at the 0.05 level, control vs. test.
**Significant at the 0.01 level, control vs. test.
() = % incidence.

The more severe grades of chronic respiratory disease were observed in lungs of some rats exposed to 5.0 ppm hydrazine and to a lesser degree in males exposed at 0.05 ppm. None of the males or the females exposed to 0.25 and 1.0 ppm showed epithelial hyperplasia. The morphological changes included peribronchial/peribronchiolar lymphoid hyperplasia, pneumonia, bronchopneumonia, and bronchiectatic abscesses. The affected animals usually showed the more severe grades of acute inflammation in the nasal cavity, larynx and/or trachea but with a higher prevalence.

The incidence of focal liver cell hyperplasia tended to be greater in treated as compared to control female rats only at the exposure levels of 1.0 ppm and 5.0 ppm. This effect was seen in female rats dying during the study and in those killed at the 2-year interim sacrifice, but it was not noted in female rats killed at the 2-1/2-year terminal sacrifice. There was no difference in the incidence of liver cell hyperplasia in treated as compared to control male rats. There was no evidence that treatment with hydrazine increased the incidence of hepatic neoplasia. It was considered, therefore, that the slightly greater incidence of liver cell hyperplasia in treated as compared to control female rats arose fortuitously and that it was not related to treatment. Acute endometritis was noted more frequently in female rats from the group receiving 5.0 ppm than in the controls or in rats from the groups receiving 0.05 ppm, 0.25 ppm, or 1.0 ppm. Acute salpingitis was present only in rats from the highest dosage group with the exception of one female from the 1.0 ppm dosage level and killed at termination.

TABLE 4. SELECTED TUMORS FOUND IN MALE FISCHER 344 RATS AFTER INHALATION EXPOSURE TO HYDRAZINE

TUMOR TYPE	Unexposed Controls (N = 149)	Exposed 0.05 ppm (N = 99)	Exposed 0.25 ppm (N = 99)	Exposed 1.0 ppm (N = 98)	Exposed 5.0 ppm (N = 99)
Nasal Cavity:					
Epithelial (Benign)	0 (0)	2 (2)	2 (2)	10 (10)**	66 (67)**
Epithelial (Malignant)	0 (0)	1 (1)	0 (0)	1 (1)	6 (6)**
Pituitary:					
Adenoma	62 (42)	31 (31)	29 (29)	27 (28)	26 (26)
Adenocarcinoma	4 (3)	0 (0)	5 (5)	4 (4)	5 (5)
Thyroid:					
Adenoma	15 (10)	5 (5)	7 (7)	9 (9)	2 (2)
Adenocarcinoma	7 (5)	6 (6)	5 (5)	9 (9)	13 (13)*
Adrenals:					
Pheochromocytoma	16 (11)	14 (14)	13 (13)	18 (18)	11 (11)
Testes:					
Interstitial cell tumor	104 (70)	80 (81)	73 (74)	83 (85)	74 (75)
Prostate:					
Squamous carcinoma	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Liver:					
Liver cell tumors	9 (6)	11 (11)	8 (8)	6 (6)	4 (4)
Lung:					
Bronchial adenoma	0 (0)	0 (0)	0 (0)	0 (0)	3 (3)
Lymphoreticular Tissue:					
Leukemias	36 (24)	20 (20)	28 (28)	22 (22)	10 (10)*
Sarcomas	8 (5)	9 (9)	3 (3)	6 (6)	3 (3)

*Significant at the 0.05 level, control vs. test.
**Significant at the 0.01 level, control vs. test.
() = % incidence.

Many microscopic variations from normal were seen in the aging mice, both control and hydrazine-exposed groups. The only lesion of significance, an increased incidence of pulmonary adenomas in the 1.0 ppm hydrazine-exposed mice, is shown in Table 5. This small increase in tumor incidence over unexposed control mice is similar to that previously reported in Swiss mice (MacEwen et al.[8]). An increased incidence of ovarian tubular adenomas was also noted in the group of mice exposed to 1.0 ppm hydrazine. This increase was not significant at the 0.05 confidence level, and its biological significance is uncertain since there was no suggestion of malignancy in this type of tumor in any of the exposed or control mice. The occurrence of nonneoplastic lesions in the C57Bl/6 mice used in this study was similar in all groups with no apparent treatment effects.

Three rodent species that inhaled hydrazine concentrations of 1.0 ppm or greater for a year developed oncogenic changes in the respiratory system. These changes appeared to be dose related in the rat in which the significant effects were epithelial tumors of the nasal turbinate. In the female rat, the tumor incidence was 4% and 37%, respectively, in animals exposed to 1.0 and 5.0 ppm hydrazine. In the male rats, the incidence was 11% at 1.0 ppm and 73% at 5.0 ppm. Nasal polyps were significant only in the 5.0 ppm hydrazine-exposed hamsters. These tumors were not seen in any unexposed control rats and in only 1 of 181 unexposed control hamsters.

A previous report of hydrazine exposures (MacEwen et al.[8]) indicated a dose-related increase in alveolargenic carcinomas in female ICR mice (a strain that normally has a high incidence) exposed to 1.0 and 5.0 ppm hydrazine. C57Bl/6 mice used in this study and exposed to 1.0 ppm hydrazine exhibited a significant increase in pulmonary adenomas. This concentration was the highest level tested in mice during the present study since the prior study had shown 5.0 ppm killed half of the mice during exposure.

A number of chronic nontumorous pathologic changes were seen in rats and hamsters exposed to hydrazine concentrations of 1.0 or 5.0 ppm. Significant differences between unexposed control male rats and the high level exposure groups occurred after one month of exposure and continued even after cessation of hydrazine treatment. Both male and female rats in the 5 ppm exposure group had a much higher incidence of upper respiratory inflammation and squamous metaplasia. Male hamsters exposed to 5 ppm hydrazine had significantly lower body weights than control animals during their exposure and the 12-month postexposure holding period. Amyloidosis, a disease frequently seen in aged hamsters, was much more prevalent in the exposed groups, and the incidence appeared to be dose related. Although the mortality rates were comparable between the test and

exposure groups of hamsters throughout the study period, there were greater numbers of changes in the hydrazine-exposed animals than their unexposed controls that are usually associated with aging such as amyloidosis and senile atrophy of the testes. Analysis of the oncogenic changes and other toxic effects of exposure to hydrazine indicates that the nononcogenic sequelae were more severe in producing debilitation and lethal effects. The oncogenic changes were mostly benign and observable only at the microscopic level producing little or no impairment of respiratory function and had no effect on life expectancy.

TABLE 5. NEOPLASTIC PATHOLOGY IN CONTROL AND HYDRAZINE-EXPOSED FEMALE C57B1/6 MICE

	Set No. 1			Set No. 2	
TUMOR TYPE	Unexposed Controls (N = 385)	Exposed 0.05 ppm (N = 364)	Exposed 0.25 ppm (N = 382)	Unexposed Controls (N = 378)	Exposed 1.0 ppm (N = 379)
Pituitary:					
Adenoma	152 (39)	94 (26)	101 (26)	109 (29)	64 (17)
Carcinoma	7 (2)	10 (3)	3 (1)	8 (2)	2 (1)
Thyroid:					
Adenoma	17 (4)	25 (7)	19 (5)	34 (9)	22 (6)
Carcinoma	2 (1)	1 (0)	1 (0)	2 (1)	1 (0)
Uterus:					
Adenocarcinoma	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)
Lymphoreticular Tissue:					
Leukemias	4 (1)	5 (2)	11 (3)	5 (1)	0 (0)
Sarcomas	145 (38)	154 (42)	150 (39)	154 (41)	139 (37)
Mammary Gland:					
All tumors	1 (0)	1 (0)	0 (0)	1 (0)	0 (0)
Liver:					
Liver cell tumor	4 (1)	9 (2)	6 (2)	6 (2)	11 (3)
Lung:					
Adenoma	8 (2)	3 (1)	5 (1)	4 (1)	12 (3)*
Adenocarcinoma	2 (1)	1 (0)	2 (1)	3 (1)	3 (1)
Ovary:					
Tubular adenoma	12/369 (3)	10/340 (3)	11/365 (3)	13/365 (4)	23/361 (6)

*Significant at 0.05 level, control vs. test.
() = % incidence

The respiratory system appears to be the primary site of hydrazine induced oncogenic changes regardless of route of administration. In studies conducted by Roe et al,[10] and others, lung tumors were induced in Swiss mice after oral administration of hydrazine in water. Lung tumors were induced in C57B1/6 mice by Mirvish et al,[11] after intraperitoneal injection. Rats given hydrazine sulfate by stomach tube by Severi and Biancifiori[12] exhibited some lung tumors. No statistically significant tumor induction was seen in rodents exposed to hydrazine concentrations of 0.25 or 0.05 ppm.

CONCLUSIONS

We conclude from these studies that hydrazine is a relatively weak tumorigen which exhibits a dose-response related tumor induction at inhaled concentrations of 1.0 ppm and 5.0 ppm. Repeated exposures to hydrazine concentrations above 5.0 ppm result in early death of rodents and dogs usually associated with malnutrition after chronic exposure.

The incidence of benign and malignant tumors was highest in nasal turbinates of rats. This rat tissue has demonstrated extreme sensitivity to the action of respiratory carcinogens (HMPA and formaldehyde) and may not be directly extrapolatable to exposure of humans who are not obligate nose-breathers. Nevertheless, the toxic and oncogenic effects seen in this study indicate that the current OSHA Threshold Limit Value of 1.0 ppm for hydrazine is unsatisfactory and is too near concentrations which cause death in chronically exposed animals. More realistically, the ACGIH recommended TLV of 0.1 ppm would be expected to provide adequate protection.

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, Washington, D.C.

Inhibition of Virus Transformation by High Energy Fuels as a Correlate of Carcinogenic Potential

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Summary

Hydrazine and naphthylamines and their derivatives were assayed for co-carcinogenic effects on ST FeSV-directed transformation of human cells. All chemicals tested at non-toxic concentrations showed anti-carcinogenic activity. The temporal relationship of chemical treatment to virus infection was more critical with the hydrazines than with the naphthylamines in that maximum anti-carcinogenic effect occurred when virus-infected cells were exposed to the hydrazines 2 hrs. post-infection, whereas the naphthylamines anti-carcinogenic effect was observed if cells were exposed either pre- or post-infection. The anti-carcinogenic effect, when compared with in vitro chemical transformation and neoplastic transformation, show a high degree of correlation. These data suggest this assay system may lend itself to a rapid screen (9-13 days) of chemicals for carcinogenic potential. Cytotoxic results showed no significant difference in shale oil or petroleum derived JP5 or DFM. Co-carcinogenic potential of JP5, JP10, RJ5, and DFM are being evaluated.

Introduction

Previous studies from this laboratory showed chemical carcinogens inhibited virus-directed feline sarcoma virus transformation of human skin fibroblast (HSF) cells at non-toxic concentrations (1). The carcinogen treatment inhibited a specific virus gene function, i.e., transformation, but not virus synthesis. Other viral gene products, RNA-dependent DNA polymerase (RDDP), Group-Specific Antigens (GSA) and Feline Oncornavirus Associated Cell Membrane Antigen (FOCMA), were detected in both carcinogen-treated or non-treated virus infected cells (2). These studies suggested the inhibitory effect on virus-directed transformation was mediated by the carcinogens, while the inhibitory effect on complete virus synthesis was cell mediated. Further, the inhibitory effect of the carcinogens was abrogated when chemicals were added to virus-infected cells 48 hrs. post-infection.

Hydrazine and its derivatives have widespread use in medicine, agriculture, and aerospace research (3). While many biologic effects of hydrazine (HZ) and its derivatives, monomethyl hydrazine (MMH), 1,1 dimethylhydrazine (UDMH), and 1,2 dimethylhydrazine (SDMH) have been studied in animals, extrapolation of these biologic effects to man has been difficult because of differential responses manifested in diverse species of test animals. Additionally, the different chemical properties of each of these chemicals has led to differential physiological responses within the same species. For example, Diwan et al. (4) concluded that genetic differences with inbred strains of mice affected the response of DMH carcinogenesis. Thus, carcinogenesis assays in rodents may lead to false negative results based on that genetic strain used in the assay. Ideally, economic, short-term, reliable in vitro assays would be invaluable in determining carcinogenic potential of chemicals.

In this study, we examined the co-carcinogenic effect of alpha-naphthylamine (ANA), phenyl-alpha naphthylamine (PANA), phenyl-beta naphthylamine (PBNA), HZ, MMH, UDMH, and SDMH on Snyder-Theilen feline sarcoma virus (ST-FeSV) transformation of HSF. The effects were further correlated with in vitro chemical transformation of HSF described by Milo and Blakeslee (3). Cytotoxic analyses of petroleum and shale oil derived jet fuels are also presented.

Materials and Methods

Cells

Human foreskin fibroblast cells (Detroit 550-CCL109, American Type Culture Collection, Rockville, MD) were grown in Minimal Essential Medium with Earles salts supplemented with 1.0 mM sodium pyruvate, 2 mM glutamine, 1% non-essential amino acids, 50 µg/ml gentamycin (Schering Diagnostics, Port Reading, NJ) and 10% fetal bovine serum (Sterile Systems, Logan, UT), thereafter designated Complete Medium (CM). Cells were serially passaged every 3-4 days at 1:2 split ratios and incubated at 37 C in 5% CO_2.

Virus

The preparation of stock ST-FeSV was described previously (6). Briefly, 10% cell-free homogenates were prepared and frozen at -85C in L15 medium and 5% FBS.

Co-Chemical Virus Assays

Preconfluent log phase growth HSF cells were trypsinized and 4×10^4 cells seeded onto 16 mm wells (Costar, Cambridge, MA) in 1.0 ml CM and incubated 18 hrs. prior to treatment. Cells pre-treated with chemicals prior to virus infection were incubated 90 min. with appropriate chemical concentration at 2, 6, or 24 hr. pre-infection. Cells were washed 2X in CM, refed and incubated at 37C until virus infection. Cells to be infected were washed with serum-free CM and treated with 0.2 ml of DEAE-Dextran (40 µg/ml (Sigma, St. Louis, MO) in serum-free CM. After 20 min., the cells were washed with CM + 5% FBS, infected with 0.05 ml ST-FeSV, diluted to 1,000 focus forming units (FFU) per ml. Twelve wells were used for each time period. Plates were rocked at 10-15 min. intervals and virus adsorbed 2 hr. at 37C. After adsorption, the inocula were aspirated and cells refed with 2.0 ml CM. Virus infected cells were treated at 2, 6, or 24 hr. following virus adsorption by incubating infected cells with designated concentration of chemical for 90 min. followed by washing and refeeding cells with 2.0 ml CM. The cells were refed with CM on the 6th day post-infection (PI), and subsequently fixed with 10% phosphate buffered formalin and stained with Giemsa 3-4 days later. Foci were counted at 25-40X with a dissecting microscope in non-treated (control) and chemically treated wells. FFU ± S.D. were determined for each treatment time and significance determined by Student's "t" test.

Chemicals

ANA, PANA, and PBNA were dissolved in spectral grade acetone at 1.0 mg/ml. Prior to use, dilutions were made in CM to experimental concentrations. HZ, MMH, and UDMH were pipetted into CM at 1 X 10^6 ppm and diluted in CM to experimental concentrations. SDMH was dissolved in 0.1-N HCl at a concentration of 10 mg/ml and diluted in CM to experimental concentrations.

Both petroleum or shale oil derived fuels were dispersed in equal volumes of 20% Triton-X (Sigma, St. Louis, MO) serum-free CM with shaking. Dilutions tested were from 5000 to 0.5 ppm. Eight wells, seeded with 500 cells/well, were treated for 90 min., washed and refed with CM supplemented with 20% FBS. Cultures were incubated 9-10 days, fixed in 10% buffered formalin, stained with Giemsa, and clones containing at least 50 cells were counted. Toxicity was determined by dividing the average number of clones in treated wells by the average number of clones in control wells. LD_{50} values were determined by plotting percent survival (ordinate) against concentration (Abcissa, log scale) on 3-cycle semi-logarithmic graph paper. LD_{50}'s were then determined by inspection.

Results

Naphthylamines

PANA treatment resulted in a dose-related suppression of transformation (Fig. 1A). Cells treated with 10 μg/ml resulted in significant inhibition of transformation at all time periods tested. Results with 20 μg/ml were similar, although treatment at 6 hrs. post-infection approached control values. Cells treated with 0.1 μg/ml showed no difference in transformation frequencies from untreated controls.

Treatment with 10 μg/ml PBNA resulted in suppression at all time periods tested (Fig. 1B). Inhibition of transformation ranged from values of 40% to 65% inhibition. However, cells exposed to 0.01 μg/ml before ST-FeSV infection resulted in enhanced focus formation at -2 hrs. whereas significant inhibition was observed when virus infected cells were treated 6 hrs. post-infection.

Figure 1C shows the effect of 10 μg and 0.01 μg/ml ANA. Both concentrations used inhibited ST-FeSV transformation, with the most significant inhibition observed when cells were treated from 2-6 hrs. pre-infection. Cells treated 6 hrs. or 24 hrs. post-virus infection had no effect on virus transformation.

Hydrazines

MMH (100 ppm and 10 ppm) significantly enhanced virus transformation in a dose-dependent manner when cells were exposed 2 hrs. pre-infection (Fig. 2A). Conversely, treatment of virus-infected cells resulted in significant inhibition of transformation when cells were treated 2 hrs. or 6 hrs. post infection. No effect was noted at 24 hrs. post-infection.

SDMH (100 μg/ml and 10 μg/ml) likewise enhanced virus transformation when cells were treated 2 hrs. pre-infection (Fig. 2B). In virus infected cells, SDMH treatment inhibited transformation by values ranging from 20% (10 μg/ml) to 30% (100 μg/ml). The inhibitory effect was observed at 2 hrs. post-infection, but not at 6 or 24 hrs. post-infection.

The effects of UDMH on virus transformation are shown in Fig. 2C. One hundred or 10 ppm exposure to UDMH inhibited transformation at 3 different time periods in relation to virus infection. UDMH inhibited transformation by values ranging from 25% to 50% when cells were exposed 6 hrs. before or 2 hrs. and 24 hrs. post-infection.

The results of HZ (60 ppm and 6 ppm) exposure are shown in Fig. 2D. Fig. 2D is a composite figure of 3 separate experiments showing HZ effects on virus transformation. Like SDMH and MMH, cells exposed to HZ 2 hrs. pre-infection, significantly enhanced virus transformation whereas exposure 2 hrs. post-virus infection significantly inhibited virus transformation as did MMH, SDMH, and UDMH.

Cytotoxicity of Fuels

Petroleum derived (PD) or shale oil derived (SOD) JP5 or diesel fuel, marine (DFM) cytotoxic analyses are shown in Table 1. The results show no significant difference in toxicity values between SOD or PD fuels. LD_{50}'s for SOD, JP5 and PD-JP5 were 102 ppm and 100 ppm, respectively, SOD-DFM and PD-DFM were 85 ppm and 87 ppm. LD_{50}'s for JP10 and RJ5 were 91 ppm and 19 ppm, respectively.

Table 1. LD_{50} cytotoxicity of shale oil and petroleum derived fuels in HSF cells.

Fuel	Derived From	LD_{50} (ppm)
JP5	Shale	102
JP5	Petroleum	100
DFM[1]	Shale	85
DFM	Petroleum	87
RJ5	Petroleum	19
JP10	Petroleum	91

[1] DFM - diesel fuel, marine

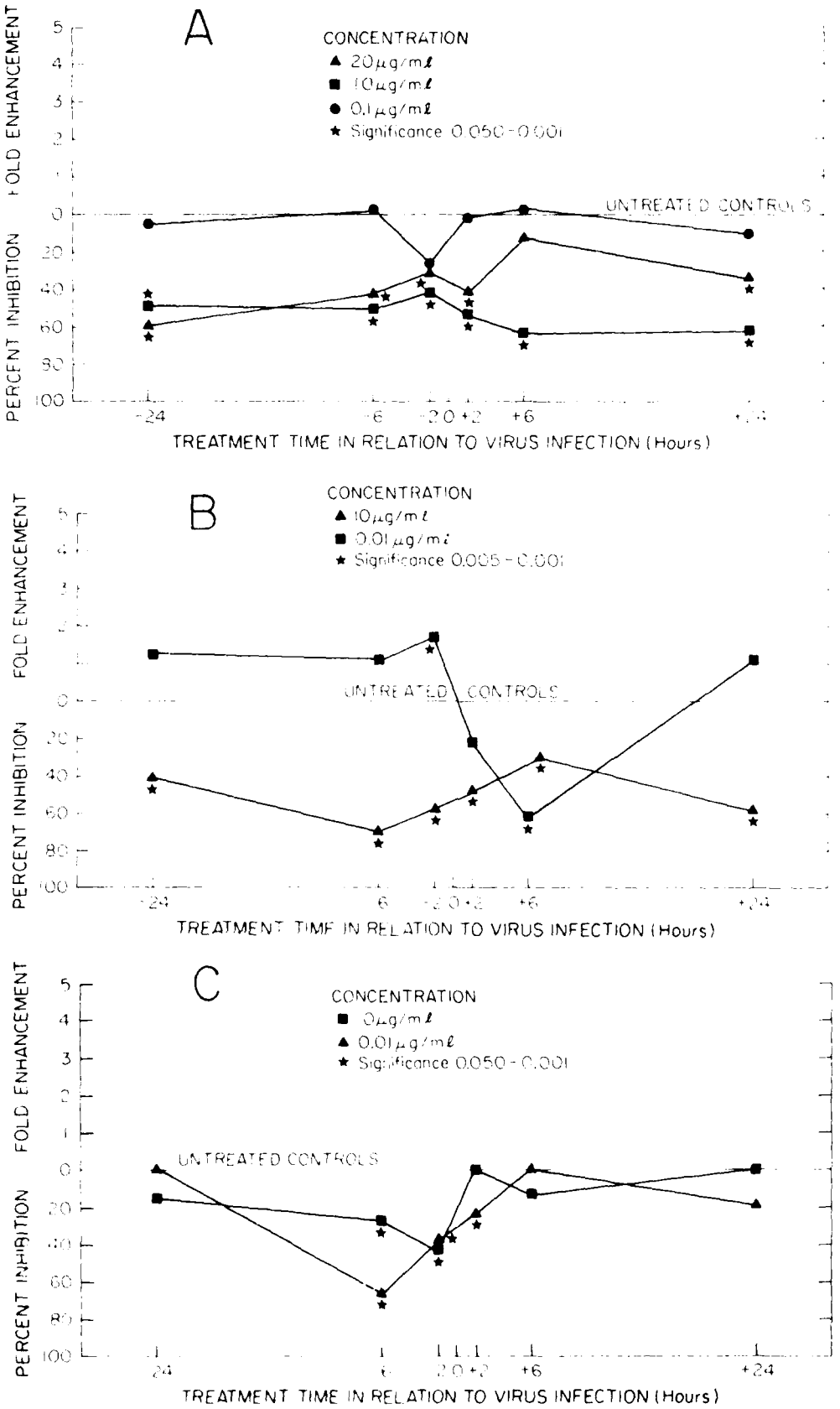

Fig. 1. HSF cells were plated in 16 mm diameter wells with 2.0 ml CM and incubated overnight. Cells were treated with ANA as described in Materials and Methods. (-) indicates cells treated before virus infection (hrs). (+) indicates cells treated after virus infection (hrs). * - significance determined by Student "t" test. A-PANA, B-PBNA and C-ANA.

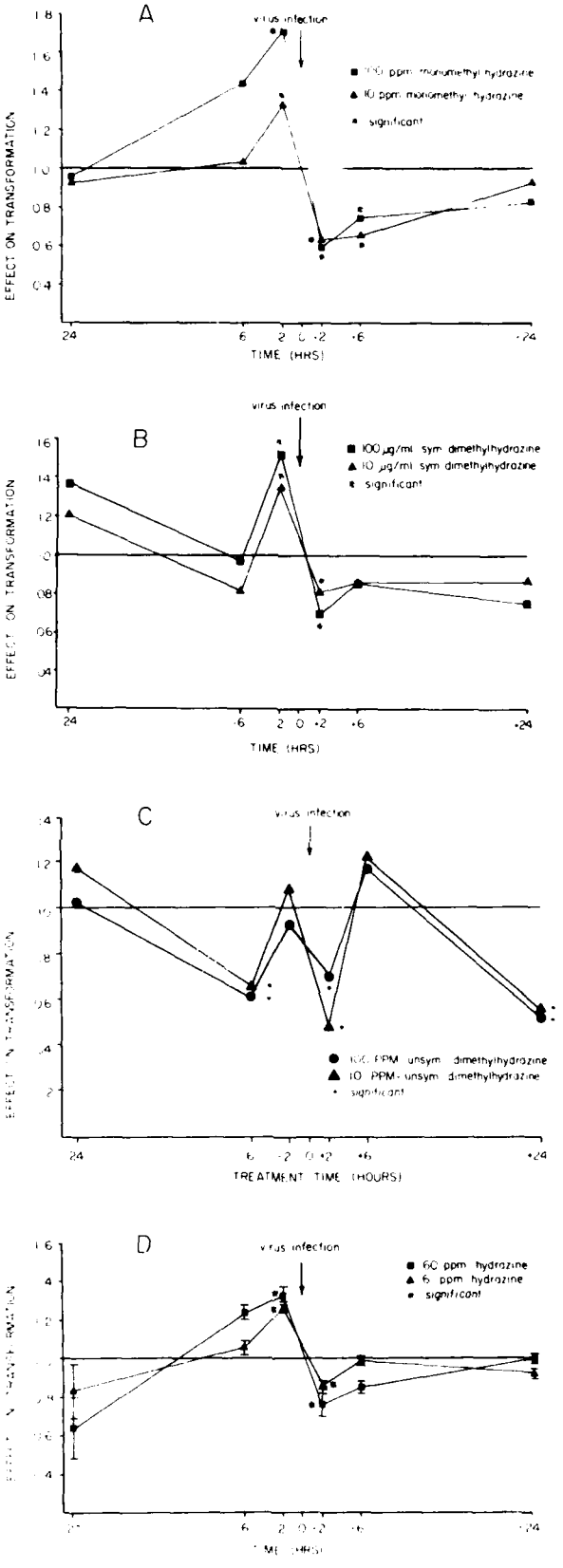

Fig. 2. HSF cells were plated in 16 mm diameter wells with 2.0 ml CM and incubated overnight. Cells were treated with ANA as described in Materials and Methods. (-) indicates cells treated before virus infection (hrs). (+) indicates cells treated after virus infection (hrs). * - significance determined by Student "t" test. A-MMH, B-SDMH, C-UDMH and D-HZ. D is a composite of 3 separate tests. Each point - n = 48 $\pm$ S.D.

Discussion

The co-carcinogenic effects of hydrazine and its derivatives and naphthylamines described in this report, when correlated with in vitro chemical transformation and neoplastic transformation, show a high degree of correlation (Table 2).

ANA, PANA, and PBNA showed 100% correlation with the two parameters, whereas MMH and SDMH showed activity in the co-carcinogenesis assays, but not in the carcinogenesis assays. HZ and UDMH, like the naphthylamines, showed 100% correlation with in vitro chemical transformation and neoplastic transformation. The inhibition of transformation from chemical treatment was not a result of cell killing in that sub-toxic concentrations were used.

Table 2. Correlation between inhibition of virus transformation, in vitro chemical transformation and neoplastic transformation.

Chemical Group	In Vitro Chemical Transformation[1]	Neoplastic Transformation[1]	Inhibition of ST-FeSV Transformation
I. Naphthylamines			
1. ANA	Yes	Yes	Yes
2. PANA	Yes	Yes	Yes
3. PBNA	Yes	Yes	Yes
II. Polycyclic Hydrocarbons			
1. BAP[2]	Yes	Yes	Yes
2. Pyrene	No	No	No
III. Hydrazines			
1. HZ	Yes	Yes	Yes[3]
2. MMH	No	No	Yes[3]
3. UDMH	Yes	Yes	Yes[3]
4. SDMH	No	No	Yes[3]

[1] Courtesy Dr. George Milo
[2] BAP - Benzo(a)pyrene
[3] Significant enhancement - 2 hrs. pre-infection

Enhanced virus transformation by HZ, MMH, and SDMH observed when cells were exposed 2 hrs. pre-infection, may be related to cell growth stimulation shown by these chemicals in dose survival studies (data not shown). Similar findings of cell stimulation have been observed with murine and feline lymphocyte cultures (7). The major effect on virus transformation (inhibition) occurred with all test chemicals when virus infected cells were exposed to the appropriate concentrations. The temporal relationship of chemical treatment to virus infection appears more critical with the hydrazines than with the naphthylamines in that maximum inhibition occurred when virus infected cells were exposed to the hydrazines 2 hrs. post-infection, whereas this inhibitory effect was observed at all 6 time periods with PANA and PBNA and at 3 time periods with ANA.

Cytotoxicity results showed no significant difference in shale oil or petroleum derived fuels. Co-carcinogenic effects with ST-FeSV are being evaluated. HZ, MMH, UDMH, SDMH, and PANA have shown mutagenic, teratogenic, or carcinogenic properties depending upon the assay used (7-11). Thus, these chemicals interact with host cell transcription or translational processes. In previous studies, we concluded the inhibitory (anti-carcinogenic) effect of benzo(a)pyrene, aflatoxin B1 or N-acetoxy-2 fluorenyl acetamide on virus transformation was not due to decreased cellular proliferation or virus synthesis (1,2). Further, the anti-carcinogenic effect was abrogated when cells were exposed >24 hrs. post-infection. The temporal relationship between infection and treatment suggested chemical interference with FeSV proviral synthesis or integration into host cell DNA.

Contrasting results have been reported on the interaction of chemical carcinogens and oncogenic RNA viruses. For example, in vivo studies showed either an anti-carcinogenic (12,13), co-carcinogenic (14,15) or no effect (16,17) on transformation depending on the virus or chemical used in the experiments. Whereas in vitro studies with rat or mouse cells showed synergism (18-20).

We previously reported anti-carcinogenic activity with three known carcinogens on FeSV transformation of human cells in vito. A recent report by Rhim and Arnstein (21) described anti-carcinogenic activity of an oncogenic murine virus on chemical-induced transformation of canine cells.

Thus, the mechanism(s) of chemical, virus, or co-chemical-virus transformation remain unknown and further studies are warranted to evaluate these interactions.

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INTERTISSUE VARIATION IN BENZO(a)PYRENE METABOLISM BY HUMAN SKIN, LUNG AND LIVER IN VITRO

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Running Title: Human Cell B(a)P Metabolism

SUMMARY

Benzo(a)pyrene [B(a)P], an environmental carcinogen, has been shown to transform human skin fibroblasts in vitro. This fossil fuel combustion product and other polynuclear hydrocarbons have exhibited a requirement to be biotransformed to their ultimate carcinogenic forms to induce transformation. B(a)P diol-epoxides are the most cited candidates as ultimate carcinogens.

When proliferating skin epithelial cells (Phase III type cells) were treated with ^{3}H-B(a)P, 83% was extractable into the organic phase as B(a)P while in the non-proliferating cells 61% occured as e parent B(a)P. Human Lung epithelial cells and liver hepatocytes were also treated with ^{3}H-B(a)P and the metabolites analyzed by HPLC. Patterns of metabolism of B(a)P by these cell populations were dissimilar to those for skin epithelial cells. In lung and liver only 9.2-21.1% remained as unmetabolized B(a)P in the non-proliferating cells, whereas 26.4% remained as B(a)P in the proliferating lung cells.

Proliferating low passage human skin fibroblasts were treated for 24 hours with ^{3}H-B(a)P after which the metabolites were removed from the growth medium with ethyl acetate. Analysis of the organic phase by HPLC demonstrated that B(a)P-tetrols (diol epoxides), B(a)P-diols and B(a)P-phenols represented a small portion (2% ea.) of the metabolites.

Our results suggest that either the ultimate form of the carcinogen is different for fibroblasts and epithelial cells or the quantitative generation of hydroxylated metabolites is not required for neoplastic transformation in fibroblast cells. If hydroxylation is required, then the site of hydroxylation may be the significant factor. Present evidence suggests that in B(a)P treated fibroblasts the activation of B(a)P in the cell takes place other than the microsomal P450 complex, presumably in the nucleus. In epithelial cells from primary target tissues, the microsomal P450 complex may play a more predominant role in the carcinogenesis process compared to the fibroblast microsomal complex.

ABBREVIATIONS

B(a)P-Benzo(a)Pyrene; MEM-minimum essential medium-Eagle; FBS-fetal bovine serum; [G^{3}H]-B(a)P-tritium-labeled benzo(a)pyrene; Ci-curie; BHT-butylated hydroxytoluene; HPLC-high performance liquid chromatography; PDL-population doubling; NFS-neonatal foreskin; HEL-human embryonic lung

INTRODUCTION

The metabolism of the environmental carcinogen benzo(a) pyrene [B(a)P] occurs in animal tissues through several pathways intended for detoxification, i.e. the mixed function oxidases or by conjugation with polar groups either sulfates, glucuronic acid or glutathione. These pathways yield a mixture of organic and water soluble metabolites, many of which have been implicated as ultimate carcinogenic forms of B(a)P (1-6).

Among the primary target tissues of B(a)P-induced carcinogenesis are the skin, lung and liver, either due to direct exposure (skin, lung) or due to an ultimate role in detoxification (liver). Metabolite profiles of B(a)P produced by cells in culture originating from any of these organs would be very informative. Since most cancers are of epithelial origin (carcinomas), the metabolism of B(a)P by epithelial cells placed in culture from these target organs would be of particular interest.

B(a)P metabolism studies are available for several rodent species (7-10). Due to the concern over human exposure, however, B(a)P metabolite profiles from human cells would be more desirable (11,12,13,14). In this report, we describe the in vitro biotransformation of B(a)P by human epidermis, peripheral lung epithelial cells and liver parenchymal cells in vitro. We compared also the B(a)P metabolite profiles of human epithelial cells with human fibroblasts.

MATERIALS AND METHODS

MATERIALS

[G-^{3}H] B(a)P (19 Ci/mmole) was purchased from Amersham Searle, Arlington Heights, IL. Synthetic B(a)P metabolite standards were received through the Chemical and Physical Carcinogenesis Branch, National Cancer Institute, Bethesda, MD.

Eagle's Minimum Essential Medium (MEM) containing 25 mM Hepes buffer was purchased from Grand Island Biological Co., Grand Island, NY. MEM-25 mM Hepes at pH 7.2 was supplemented with sodium pyruvate (1.0 mM), glutamine (2.0 mM), nonessential amino acids (1X) and vitamins (1X) (15). All of these supplements were obtained from M.A. Bioproducts, Walkersville, MD). The MEM also contained sodium bicarbonate (0.2%) and gentamycin (5µg/ml).

Fetal bovine serum (FBS) was purchased from Reheis Chemical Co., Kankakee, IL. Collagenase was obtained from Worthington Biochemical Corp., Vineland, NJ, Instagel scintillation cocktail from Packard Instrument Co., Downers Grove, IL, and methanol (MCB OmniSolv) for HPLC from Curtin Matheson Scientific, Inc., Cleveland, OH.

METHODS

Primary Skin Epithelial Cell Cultures

Primary cultures of human neonatal foreskin epithelial cells were established as described previously (16-18). This method involves an initial digestion of the tissue with collagenase (16), followed by selective detachment of fibroblasts with trypsin after the primary culture was established (17,18).

Primary Lung Epithelial Cell Cultures

Human fetal lung tissue obtained from William J. Douglas (Tufts University) was enzymatically dispersed for 4 hours with 0.25% collagenase in MEM supplemented with 20% FBS. The digestions were done at 37 C in a 4% CO_2 enriched air environment. The cells were centrifuged at 650 x g for 10 minutes, washed with MEM, and resuspended in MEM containing 20% FBS. The cells were then seeded at a concentration of 20,000 cells/cm^2. After 2 hours, the residual lung cells in suspension were removed, and the cells attached to the substratum were fed with MEM containing 40% FBS.

The primary lung cell cultures contained less than 10% fibroblasts when the B(a)P metabolism studies were undertaken. They were composed of mixtures of epithelial cell types. However, the major portion of the population contained lamellar structures, tonofilaments, and desmosomes as determined by electron micrsocopy. At this time, we have designated these cultures as mixed peripheral lung epithelial cell populations.

Primary Liver Parenchymal Cell Cultures

Normal adult human liver was obtained from the Tumor Procurement and Pathology Laboratory, Comprehensive Cancer Center, Ohio State University. Liver from surgery was placed immediately into culture by a modification of the method of Schaeffer and Kessler (19). The tissue was minced into 1 to 2 mm pieces and incubated at 37 C for 6 hours in the presence of 0.25% collagenase in MEM containing 20% FBS. Following the incubation, FBS was added to a final concentration of 50% and the parenchymal cells were selectively pelleted by centrifugation at 650 x g for 3 minutes. The cells, were resuspended in MEM containing 20% FBS and insulin (0.5 U/ml), and were seeded into 25 cm^2 tissue culture flasks at a c ncentration of 15,000 cells/cm^2. Following a 4 hour attachment period at 37 C in a 4% CO_2 enriched air environment, the cultures were washed to remove debris. The cultures were fed with MEM containing 20% FBS, and a confluent primary culture of parenchymal cells was obtained within 72 hours. The cultures were comprised of greater than 95% liver parenchymal cells.

Primary Skin Fibroblast Cell Cultures

Human neonatal foreskin fibroblasts were grown, serially subpassaged and treated with B(a)P as described previously (2).

[G-^{3}H] B(a)P Treatment

Treatment of cells with [G-^{3}H] B(a)P (0.105µM at 1 mCi/ml, 19 Ci/mmole) was accomplished after dissolving the compound in spectral grade acetone. The final solutions were added to MEM containing 10% FBS at 37°C. The [G-^{3}H] B(a)P-containing medium (3.3 ml/25 cm^2 flask) was used to feed the cultures, after which they were incubated at 37°C in a 4% CO_2 enriched air environment (20). All procedures with [G-^{3}H] B(a)P were carried out under yellow light.

B(a)P Metabolite Extraction

Twenty-four hours after the administration of radiolabeled B(a)P, aliquots of the growth medium were removed and partitioned with 3 volumes of ethyl acetate containing the antioxidant butylated hydroxy toluene (BHT, 0.8 mg/ml). The phases were separated, and the organic phase was passed over anhydrous sodium sulfate, filtered, dried under argon, and stored at -20 C. The sample was dissolved in 200µl methanol, and centrifuged at 12,000 x g for 2 minutes to remove particulate matter prior to analysis by HPLC.

HPLC Analysis

The solvent delivery system consisted of a Beckman (Beckman Instruments, Inc., Irvine, CA) Model 322 MP programmable HPLC with CRIA integrating printer plotter. The system was equipped with a Model 153 fixed-wavelength UV (254 nm) detector, and fractions were collected directly in scintillation minivials with an LKB Model 2112 fraction collector. The reversed phase column utilized was a Beckman Ultrasphere-ODS (150 x 4.6 mm).

The column was equilibrated with 85% methanol in water, and the flow rate was 1 ml/minute throughout the analysis. A 20 µl sample was injected, and elution was initiated with a mobil phase of 85% methanol in water. After 30 seconds, the methanol concentration was increased to 100% over a period of 1.5 minutes. Aliquots of 0.2 ml were collected in each minivial and 2 ml of Instagel scintillation cocktail was added. Radioactivity was measured with a Beckman LS-9000 scintillation counter. After complete elution of the hydrocarbons, the column was re-equilibrated with 85% methanol for 8 to 10 minutes. Authentic B(a)P metabolite standards were detected by UV absorbance at 254 nm.

RESULTS

Representative cultures of human peripheral lung epithelial cells (HEL), skin epithelial cells, and liver parenchymal cells are shown in Figure 1. The lung (Figure 1A) and liver (Figure 1C) cells attach to the substratum, and they will each grow to a confluent monolayer. The skin epithelial cells (Figure 1B) have the additional capability of being able to grow in a vertically stratified layer. For our purposes, proliferating cell cultures are those that have significant areas of the substratum (usually 40-50%) not covered with cells, while non-proliferating cultures are confluent. The skin epithelial cells exhibit vertical stratification under both circumstances. The proliferative states of duplicate cultures were verified by ^{3}H-thymidine-labeling and autoradiography (data not shown).

The conversion of [G-^{3}H]B(a)P to water soluble metabolites by human epithelial cells derived from lung, skin and liver is presented in Table 1. The cells from lung and liver generated significantly greater amounts of the water soluble metabolites than the skin epithelial cells.

The ethyl acetate-soluble metabolites of B(a)P generated by proliferating HEL epithelial cells is depicted in Figure 2. The HPLC data indicates that less than 30% of the organic-soluble material is accounted for by the parent hydrocarbon B(a)P. The major portion of the radioactivity (34.3%) was localized under the B(a)P-tetrol peak. The relative distribution of ethyl acetate-soluble metabolites is summarized on line one of Table 2.

A significant difference in the HPLC metabolite profile of non-proliferating HEL epithelial cells can be seen in Figure 3. Although there is little difference in the amount of B(a)P-tetrols produced by proliferating, (Figure 2) and non-proliferating (Figure 3) HEL cells, a major polar derivative peak is predominant in the latter. In addition, a B(a)P-diol peak in Figure 3 is almost non-existent. The early eluting polar component produced by non-proliferating HEL cells is made up almost totally of B(a)P sulfate conjugates because this peak is reduced greater than 80% by treatment with arylsulfatase (data not presented). Again, a summary of relative B(a)P-metabolite production by the non-proliferating HEL cells is presented in Table 2. There is a decrease in the amounts of both B(a)P diols and phenols when compared to proliferating HEL cells, and more of the B(a)P is metabolized.

Proliferating NFS epithelial cells metabolize much less B(a)P than HEL cells (Table 2). After 24 hours, 83.9% of the ethyl acetate-extractable hydrocarbon is the parent compound B(a)P. None of the B(a)P-metabolites account for more than 4.5% of the radiolabeled material.

Non-proliferating NFS epithelial cells metabolize more than twice as much B(a)P than proliferating NFS cells (Table 2). As with HEL cells, the major increase is in the synthesis of polar derivatives. The confluent NFS culture gives rise to somewhat more B(a)P-tetrol, but there is little change in B(a)P diols and phenols.

Profiles of intracellular distribution of B(a)P-metabolites (foreskin fibroblasts) revealed that a major portion of the B(a)P remains in its parent form, (21). Extracellular oxygenated metabolites account for less than 10% of the added B(a)P after 24 hour treatment, Figure 3. B(a)P-tetrols, diols and phenols accounted for less than 1% of the remaining metabolites.

Liver parenchymal cells actively metabolize B(a)P similar to HEL epithelial cells (Table 2). Only 21.1% of the organic soluble hydrocarbon is unmetabolized B(a)P after 24 hours. Polar derivatives (26.3%) and B(a)P-tetrols (25.2%) are found in significant amounts in the medium of the confluent liver cell culture. Both levels are similar to those in the confluent HEL epithelial cell culture and higher than the NFS epithelial cell culture.

DISCUSSION

Cell proliferation is required for the fixation of the carcinogenic event. We have been able to induce neoplastic transformation of normal human fibroblasts with a variety of chemicals, including B(a)P, if the cells are in an enhanced proliferative state (2,15). However, we have not been able to transform confluent, non-proliferating human cells even if the cells are subpassaged and allowed to divide immediately after the carcinogen treatment.

Even though cell proliferation is required for chemical carcinogen-induced neoplastic transformation, most studies of B(a)P activation make use of non-proliferating cell cultures (3,4,7,11,13,14,22). It has been shown with human skin fibroblasts, that stationary (non-proliferating) cultures established by seeding cells at a low density in nutrient-deficient medium yield 10 times more oxygenated B(a)P-metabolites than proliferating cultures (4). The significance of these oxygenated B(a)P metabolites to carcinogensis is unknown. We reported previously that non-confluent skin fibroblasts transport B(a)P to the nucleus while confluent fibroblast do not (2,20). Therefore, the increased metabolism by non-proliferating cultures may be without effect.

Human epithelial cells exhibit different growth characteristics in vitro when compared to fibroblasts, and most human cancers are of epithelial origin (carcinomas); therefore, we felt it was of interest to examine the metabolism of B(a)P, by epithelial cells under proliferating conditions which favor transformation and under non-proliferating conditions which do not favor transformation. Epithelial cells from different primary target tissues were utilized to assess intertissue variation.

The lung and liver cell cultures produce more water soluble metabolites than do the skin cells (Table 1). The water-soluble derivatives consist mainly of detoxified conjugates of B(a)P (13,22). Considering that the epidermis is an effective physical barrier, the need for an active detoxification pathway may be less important than with lung and liver.

B(a)P diol-epoxides are considered to be the ultimate carcinogenic metabolites of B(a)P (1,23). The metabolic activation of B(a)P occurs at the P450 locus in the plasma membrane, and the B(a)P-diol-epoxides synthesized form adducts predominantly with the N^{2} moiety of guanine in nucleic acids (21-23). Such interactions with DNA are thought to be responsible for the induction of carcinogenesis.

In an aqueous environment, B(a)P-diol-epoxides are rapidly hydrolyzed to B(a)P-tetrols, so these are the most significant products one can measure in the ethyl acetate phase. Little change was observed in the generation of B(a)P-tetrols by proliferating and non-proliferating human epithelial cell cultures (Table 2). This indicates that the activation pathway is functioning under both circumstances, and that the ultimate carcinogenic B(a)P-diol-epoxides are synthesized.

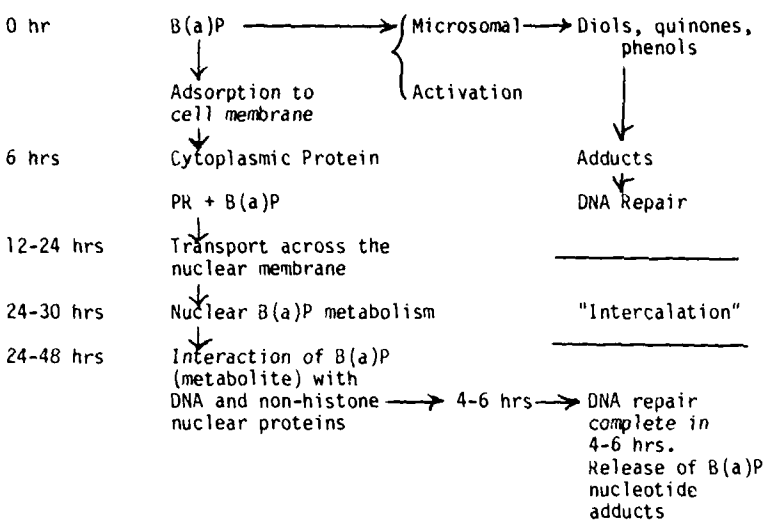
The early eluting polar B(a)P metabolite peak (Figure 3; Table 2) is composed predominantly, if not totally, of B(a)P sulfate conjugates. Extraction of these detoxification products of B(a)P phenols with ethyl acetate has been reported using a different chromatographic system (7,24). Our results for human epithelial cells are consistent with this detoxification pathway being activated in non-proliferating cells. With the HEL cells (Figure 2 and 3; Table 2), the increase in the early polar derivative(s) in the confluent culture is accompanied by a decrease in B(a)P-phenols and diols.

The results presented in this manuscript demonstrate that human lung epithelial cells and liver parenchymal cells biotransform B(a)P to a much greater extent than do human skin epithelial cells. The increased biotransformation involves both the activation and the detoxification pathways. In addition, non-proliferating epithelial cells have a greater propensity for deactivating B(a)P than do proliferating epithelial cells. This may help explain the need for cell proliferation during carcinogen exposure in order to obtain transformed cells in vitro.

Transformable fibroblast cell populations (2) produce less than 3% hydroxylated-epoxide metabolites (oxygenated forms), (21). In excess of 98% of the B(a)P remains as B(a)P.

The intracellular distribution of B(a)P in the fibroblasts appears to occur as B(a)P bound to a low molecular weight lipoprotein (21). Our present DNA adduct data (Tejwani, Jeffery and Milo, unpublished data) suggests that the ultimate major carcinogenic form in the nucleus is Benzo(a)Pyrene 7,8 diol 9,10 epoxide -1 (anti)-deoxyguanosine . This adduct has been reported by others to be the major adduct excised by the error free repair system from the DNA, (25,26). In fibroblasts, activation by the microsomal P450 complex is not necessary to biotransform B(a)P to an oxygenated carcinogenic derivative, however activation must occur prior to induction of carcinogenesis. We conclude from these data that the oxygenation of the B(a)P to the carcinogenic metabolite must take place in another intracellular location, presumably the nucleus.

A proposed mechanism for the biotransformation of B(a)P in fibroblasts followed by the induction of a carcinogenic event is presented here.



Similar studies are in progress with human epithelial cells from target tissues to describe B(a)P activation and induction of a carcinogenic event.

Table 1

Partitioning of Radioactivity After Incubation of Human Epithelial Cells with [G-^{3}H]B(a)P for 24 Hours

Cells	Percent Organic-soluble	Water-soluble
HEL		
Proliferating	60	40
Non-proliferating	52	48
NFS		
Proliferating	77	23
Non-proliferating	81	19
Liver		
Non-proliferating	42	58

TABLE 2

B(a)P Metabolites Produced by Human Epithelial Cells After Incubation with [G-^{3}H]B(a)P for 24 Hours[a].

Cells	Polar Derivatives[b]	B(a)P Tetrols	B(a)P Diols	Unidentified Metabolite[c]	B(a)P Phenols	B(a)P
HEL						
Proliferating	---	34.3	13.7	9.5	12.2	26.4
Non-proliferating	32.1	27.5	2.0	6.9	3.8	9.2
NFS						
Proliferating	1.9	4.5	1.2	3.4	2.1	83.9
Non-Proliferating	17.0	9.4	1.8	3.0	1.8	60.5
Liver						
Non-proliferating	26.3	25.2	3.5	11.6	3.2	21.1

[a]The values are expressed as percent of total radioactivity, and they represent the integrated values under the HPLC peaks.

[b]Treatment with aryl sulfatase removes greater than 80% of this component indicating it is predominantly a sulfate conjugate of B(a)P.

[c]B(a)P-6,12-dione and 6-hydroxymethyl-B(a)P co-elute at this position.

ACKNOWLEDGMENT

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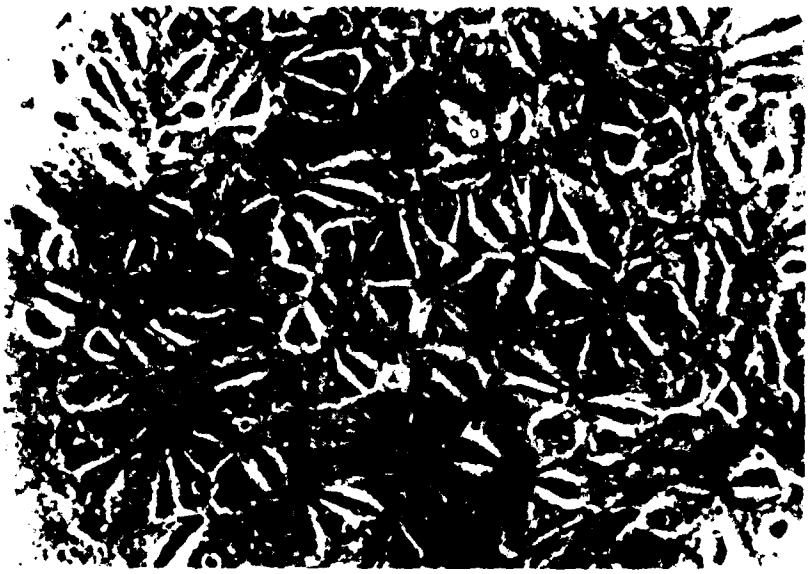
Figure 1

A. Human peripheral embryonic lung cells at passage 1 at saturation density state of growth, 100 X.



B. Human foreskin epidermal cells at a saturation density state of growth at passage 1, 100 X.

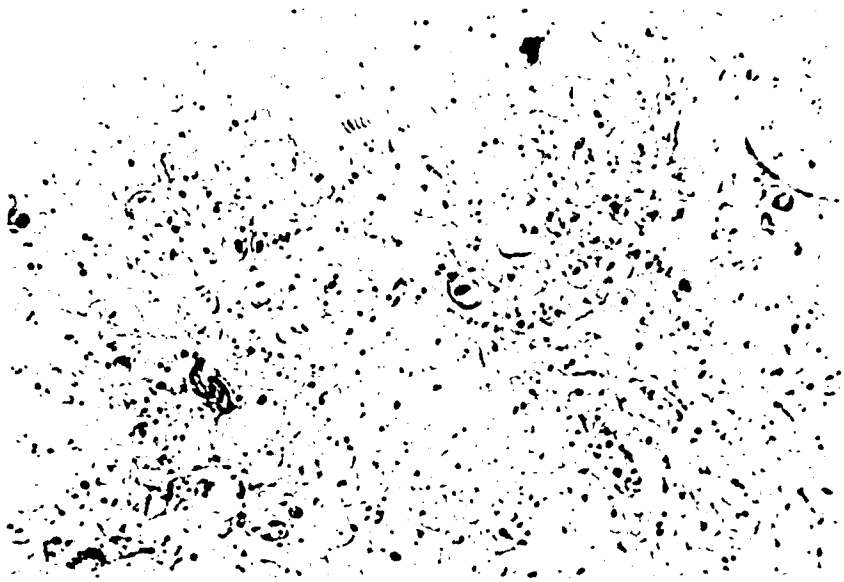
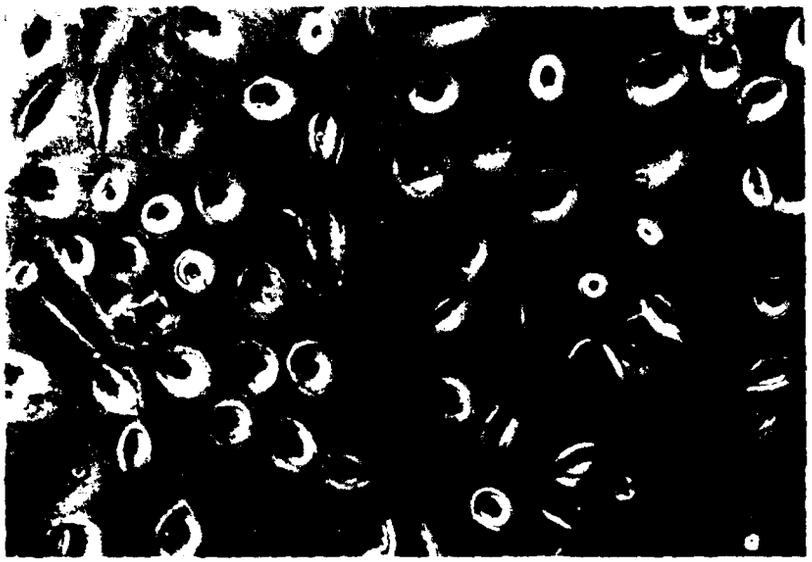


Figure 1

C. Human embryonic liver at passage 2 at a saturation density state of growth, 160 X.



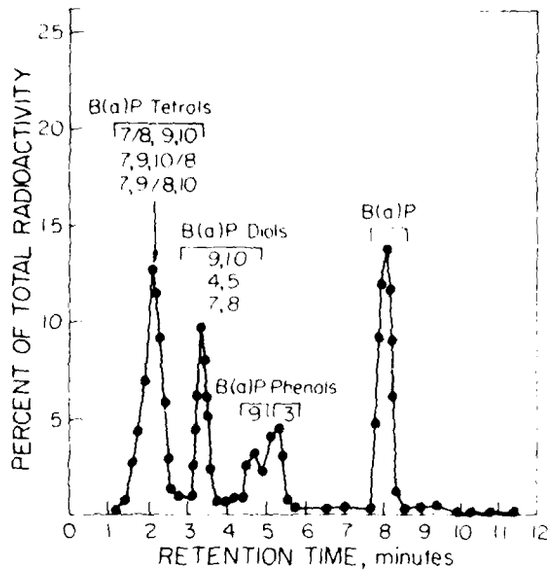
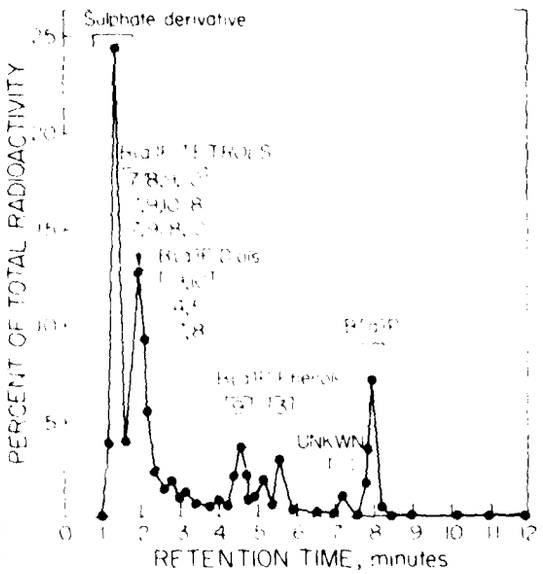


Figure 2. Metabolite profile of B(a)P produced by proliferating HEL cells. The activity is expressed as a percent of the total radioactivity of B(a)P plotted as a function of the retention time on the HPLC column .

Figure 3. Metabolite profile of B(a)P produced by HEL cells at a confluent density. The activity is expressed as outlined in Figure 2, legend.



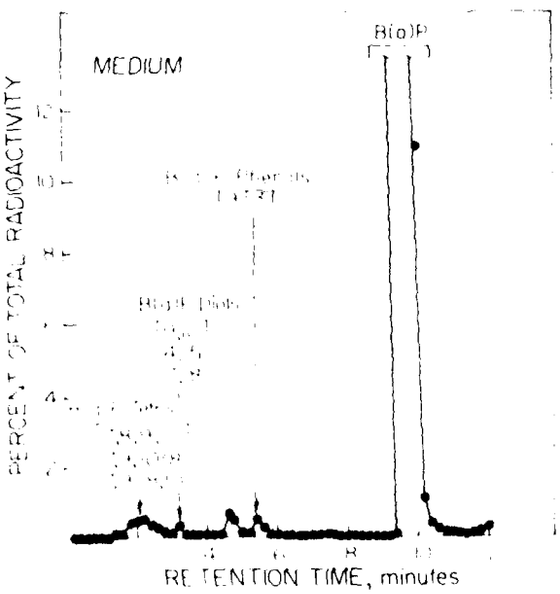


Figure 4. Metabolite profile of B(a)P produced by proliferating ,transformable foreskin fibroblasts. The activity is expressed as outlined in Figure 2, legend.

TOXICITE DES GAZ DE DECOMPOSITION THERMIQUE DES MATIERES COMBUSTIBLES

PROTOTYPE DE CHAMBRE D'ESSAIS

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Lorsque le feu se déclare dans une enceinte close, telle qu'une cabine d'avion, la survie par réaction de fuite n'est pas immédiatement possible et il faut d'abord regagner le sol dans les conditions vitales minimum. Dans ce cas, les gaz toxiques deviennent le problème majeur. C'est pourquoi il est nécessaire de pratiquer une sélection des matériaux présentant le minimum de toxicité en cas de feu à bord.

Nous avons été amenés à définir une chambre d'essais permettant de maîtriser suffisamment les paramètres physiques de la décomposition thermique des matériaux étudiés pour rendre le plus reproductible possible les atmosphères produites. Ce prototype doit posséder les caractères spécifiques suivants : volume clos relativement faible, renouvellement d'air rapide (total en trois minutes), possibilité d'arrêt pratiquement total de la ventilation, pour pouvoir tenter la simulation de situations rencontrées en aéronautique.

Nous présentons ici le prototype de chambre d'essais réalisé au Centre d'Etudes et de Recherches de Médecine Aérospatiale ainsi que les premiers résultats obtenus. Le but de cette étude est de pouvoir, ultérieurement, et suivant les critères de danger choisis, fournir les bases de sélection de matériaux d'aménagement de cabines d'aéronefs.

INTRODUCTION

Une série d'évènements récents a de nouveau attiré l'attention sur le drame que constitue un incendie dans les lieux restreints et habités. Dans les constructions terrestres les individus soumis à un incendie peuvent espérer s'y soustraire par une évacuation des locaux si les conditions sont favorables. Par contre, lorsque le feu se déclare dans une enceinte close telle qu'une cabine d'avion, la réaction de fuite n'est pas possible et il faut d'abord regagner le sol dans les conditions minimales de survie. Un incendie en vol (feu de moteur, feu de cabine) est relativement rare. Selon l'AGARD (1) il ne compte que pour un vingtième de la probabilité des accidents sérieux comparé aux incendies après crash. Ce type d'incendie est généralement bien contrôlé, surtout en ce qui concerne les feux de moteur. En ce qui concerne les feux de cabine, une étude de la C.A.A. (2) leur attribue 7 accidents en vol dans l'aviation civile entre 1963 et 1974. Les trois principaux ont cependant fait au total 251 victimes. On peut donc dire que cette éventualité est rare et que si les passagers doivent survivre, le feu ne peut être que de nature restreinte. Mais la contribution des matériaux de cabine à l'aggravation des conditions d'incendie suivant certains atterrissages catastrophiques est loin d'être négligeable. De ce fait, les dangers dûs aux feux de matériaux de cabine ont été placés au quatrième rang sur les sept de l'échelle établie par l'AGARD (1). Ces matériaux, surtout depuis le développement massif de l'emploi des matériaux synthétiques, et l'ensemble des combustibles divers (vêtements, journaux, revues, alcools, bagages à main) apportés par les passagers sont très sensibles au feu. Dans ce cas, les gaz toxiques deviennent le problème majeur.

C'est pourquoi il est nécessaire de pratiquer une sélection de matériaux présentant le minimum de toxicité en cas de feu à bord. Dans cette optique, de nombreuses études ont été menées à travers le monde -et principalement aux Etats-Unis- pour évaluer la toxicité des produits de décomposition thermique de divers matériaux (3 à 21).

Ces diverses études conduisent à deux remarques essentielles :

- les produits de décomposition thermique des matériaux varient énormément en fonction de nombreux facteurs : nature et composition du matériau, température de décomposition, composition de l'atmosphère, conditions de ventilation, géométrie des échantillons étudiés...
- l'étude analytique des atmosphères produites ne suffit pas pour évaluer les risques encourus en cas d'intoxication accidentelle. L'introduction d'animaux dans le processus expérimental est nécessaire pour estimer la perturbation des paramètres physiologiques au cours de cette intoxication.

Nous avons tenu compte de ces deux remarques dans l'étude concernant l'évaluation de la toxicité des produits de décomposition thermique des matériaux d'aménagement des cabines d'avion que nous avons entreprise sur commande de la Direction des Recherches Etudes et Techniques (D.R.E.T.).

Nous avons été amenés :

1 - A définir un "modèle feu" permettant de maitriser suffisamment les paramètres physiques de la décomposition thermique des matériaux étudiés pour rendre le plus reproductible possible les atmosphères produites. Du fait que dans les aéronefs on a souvent à faire à des conditions extrêmes de feu : combustion avec excès d'air, éventuellement oxygène, ou , au contraire, suivant

la localisation (soute, placard à vêtements) ou en cas d'arrêt de la ventilation, à des conditions de décomposition thermique voisines de la pyrolyse avec confinement de l'atmosphère, ce système doit, de plus, posséder les caractéristiques spécifiques suivantes :

. renouvellement d'air rapide (total en trois minutes) par flux laminaire ;
. possibilité d'arrêt pratiquement total de la ventilation ;
. possibilité d'apport d'oxygène pur dans la ventilation ;
. volume clos relativement faible.

2 - A exposer des animaux aux gaz toxiques produits afin d'étudier la réponse à l'agression de l'organisme entier avec ses susceptibilités et ses réactions.

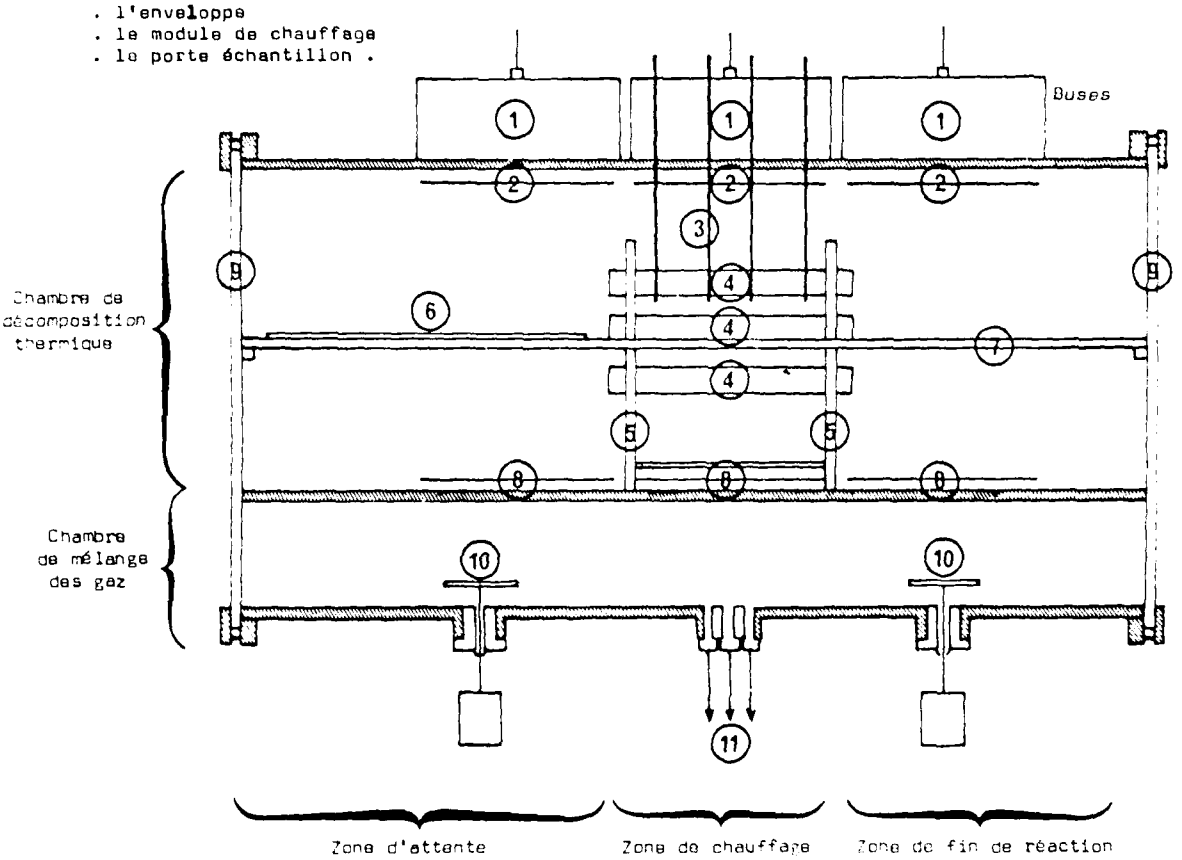
Le système expérimental présenté dans cet article comprend donc un "modèle feu" original dont les caractéristiques permettent de répondre à des contraintes spécifiquement aéronautiques dans le cadre de notre étude mais peuvent également autoriser son emploi pour simuler les conditions de feu dans des constructions terrestres ou autres atmosphères confinées (sous-marins, navires, véhicules stratégiques divers...). Ce "modèle feu" est relié à :

. un ensemble de dosage des composants de l'atmosphère créée ;
. un ensemble d'exposition d'animaux pour l'évaluation des effets physiologiques de ces gaz.

DESCRIPTION GENERALE DU "MODELE FEU"

Le "modèle feu" est composé de trois parties distinctes :

. l'enveloppe
. le module de chauffage
. le porte échantillon .



SCHEMA N° 1

1.1. - L'enveloppe

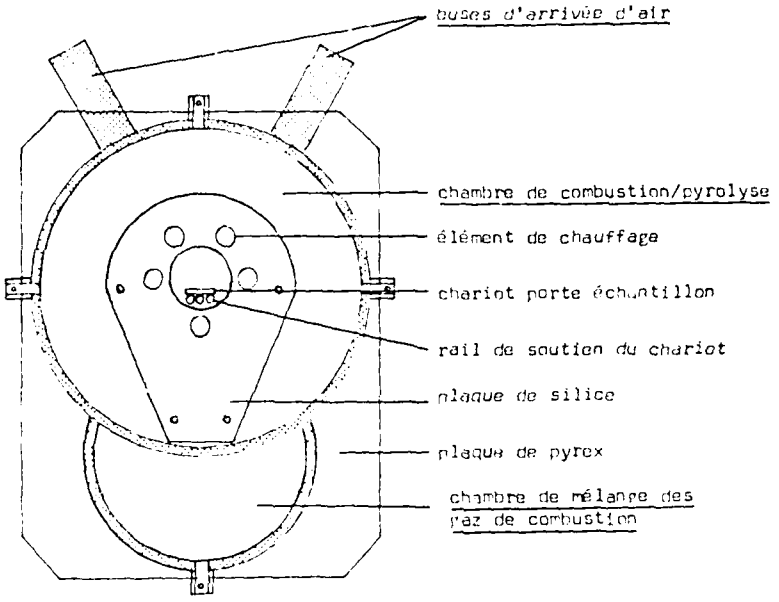
Elle est réalisée en acier Inox 316 Titane, alliage présentant une bonne inertie chimique et une résistance élevée aux gaz corrosifs dégagés lors des décompositions thermiques.

L'enveloppe du "modèle feu" comprend trois parties solidaires :

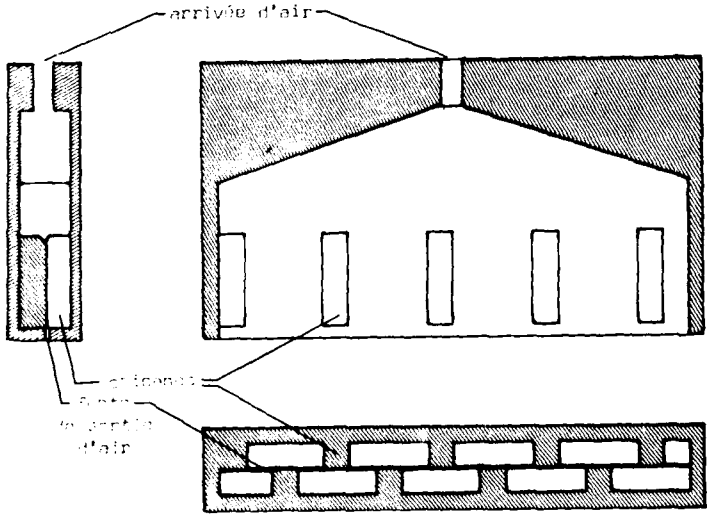
. la chambre de décomposition thermique ;
. les buses d'admission d'air ;
. la chambre de mélange des gaz de combustion.

1.1.1. - La chambre de décomposition thermique

Cette chambre, d'un volume total de 30 litres, partie centrale du "modèle feu", est constituée par un cylindre de 850 mm de longueur et de 300 mm de diamètre intérieur ouvert à ses deux extrémités pour permettre un accès aisé en son centre (cf schémas n° 1 et 2). Lors des expériences



SCHEMA N° 2



SCHEMA N° 3

de décomposition thermique, l'étanchéité de cette chambre est assurée par deux plaques de Pyrex (9) maintenues par des supports métalliques.
Cette chambre comporte dans sa partie supérieure deux séries de trois fentes (2) chacune ayant une longueur de 180mm et une largeur de 1mm. Ces six fentes supérieures constituent les arrivées d'air dans la chambre de décomposition thermique. A la partie inférieure de cette chambre, deux séries de trois fentes (8) ayant chacune une longueur de 180mm et une largeur de 2mm permettent l'évacuation des produits et gaz de décomposition thermique vers la chambre de mélange des gaz.

1.1.2. - Les buses d'admission d'air (cf. schéma n° 3)

Les buses d'admission d'air sont au nombre de six et correspondent aux six fentes d'arrivée d'air dans la chambre de décomposition thermique. Chaque buse est constituée d'une pièce métallique de 180mm de longueur et de 30mm de largeur. A l'intérieur, les buses comportent un système de chicanes destinées à répartir l'air de façon uniforme sur la longueur totale de chaque fente.

1.1.3. - La chambre de mélange des gaz de combustion

Cette chambre, située sous la chambre de décomposition thermique, est en relation avec celle-ci par l'intermédiaire de six fentes permettant le passage des gaz de combustion. Elle est constituée par un demi cylindre de 850mm de longueur et de 90mm de rayon soudé à la partie inférieure de la chambre de décomposition thermique.

1.2. - Le module de chauffage

Le chauffage du "modèle feu" est assuré par un ensemble de cinq résistances électriques chauffantes de 600 W chacune, soit une puissance maximale de 3 KW.

Le module de chauffage est constitué de deux plaques de silice (5) solidaires l'une de l'autre par quatre entretoises en silice. Ces deux plaques, distantes de 180mm, sont percées d'un trou de 64mm de diamètre permettant le passage de l'échantillon et de cinq trous latéraux de 25mm de diamètre. Ces cinq trous sont destinés à recevoir cinq tubes de quartz (4) à l'intérieur desquels sont placées les résistances chauffantes ainsi protégées des gaz corrosifs dégagés lors des expériences de décomposition thermique.

Ce module de chauffage est placé dans la partie centrale de la chambre de décomposition thermique.

1.3. - Le porte échantillon

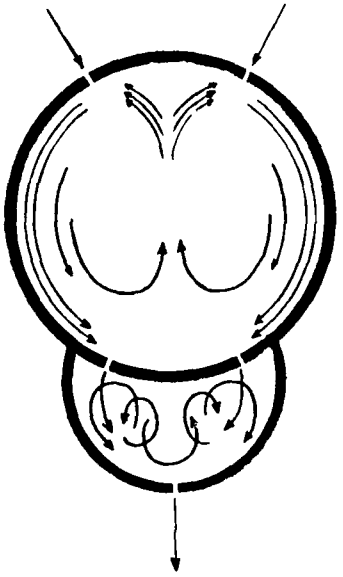
L'échantillon du matériau testé est placé sur un chariot en quartz (6) de 300mm de longueur et de 30mm de largeur. Ce chariot est mobile et posé sur deux rails en quartz (7) traversant la chambre de décomposition thermique sur toute sa longueur. Ces deux rails, solidaires entre eux, reposent à chaque extrémité de la chambre sur des barres métalliques de soutien.

CARACTERES SPECIFIQUES DU "MODELE FEU"

2.1. - La ventilation

L'air arrivant dans la chambre de décomposition thermique est réparti par les six buses d'admission d'air (1). Le débit global est réglé à l'aide de six débimètres à 1200 litres/heure. Ce réglage assure un renouvellement de l'air de la chambre de décomposition thermique toutes les trois minutes, ce qui correspond aux conditions aéronautiques normales de ventilation. Le débit d'air et la teneur en oxygène peuvent être modifiés pour étudier l'influence de ces deux facteurs sur la décomposition thermique des échantillons étudiés.

Les buses d'admission d'air (1) ont été conçues avec un système de chicanes permettant d'obtenir à l'intérieur de la chambre de décomposition thermique, un flux d'air laminaire (cf. schéma n° 4).



SCHEMA N° 4

Les gaz de combustion passent ensuite dans la chambre de mélange ou un ensemble de deux agitateurs (10) assure l'homogénéité du gaz à analyser.

2.2. - Le système de régulation et de programmation de température

Le chauffage de l'échantillon est réglé par un système de régulation et de programmation de température piloté par un des quatre thermocouples (3) traversant l'enveloppe.

2.2.1. - Le régulateur de température

Il commande l'alimentation électrique du système de chauffage et assure une stabilisation de la température à la valeur affichée.

2.2.2. - Le programmateur de température

Ce générateur délivre une tension de référence qui croit ou décroit linéairement pendant une durée prédéterminée allant de une minute à quelques heures.

A l'aide de cet ensemble de régulation et de programmation de température, nous pouvons donc effectuer deux types d'essais de décomposition thermique :

- essais à température fixe pendant un temps déterminé;
- essais avec montée progressive de température.

Dans les conditions actuelles, avec une puissance de 3 KW, la température maximale obtenue dans la zone de chauffage est de 650° C. Une modification des résistances chauffantes et une augmentation de puissance doivent permettre d'atteindre prochainement une température de 900° C.

2.2.3. - Le système d'avance de l'échantillon

Les matériaux à tester sont placés sur le chariot porte échantillon qui peut se déplacer sur toute la longueur de la chambre de décomposition thermique. Ce chariot, supporté et guidé par deux rails en quartz, est tiré grâce à un fil de platine relié à un moteur électrique à allure variable. Il est possible de ce fait d'effectuer deux types d'essais :

- essais statiques : l'échantillon reste centré dans la zone de chauffage pour subir une décomposition thermique à température fixe ou en programmation de température.
- essais dynamiques : l'échantillon est placé avant le début de l'expérience dans la zone d'attente. Lorsque la température désirée est atteinte, le moteur d'entraînement du chariot est mis en marche. Celui-ci avance donc dans la zone de chauffage à vitesse constante. Nous réglons actuellement l'avance du chariot à une vitesse de 1 cm/mn. En testant des échantillons d'une longueur de 30 cm, nous réalisons donc des essais d'une durée totale de 30 minutes.

Un système d'asservissement de l'avance du porte échantillon en fonction de la vitesse de combustion sera décrit ultérieurement.

SYSTEMES D'ETUDES DES PRODUITS DE DECOMPOSITION THERMIQUE

L'étude des produits de décomposition thermique comporte une partie analytique et une partie toxicologique.

L'analyse de l'atmosphère produite dans la chambre de décomposition thermique est assurée par un ensemble d'appareils connectés à la sortie de la chambre de mélange des gaz (cf. schéma n° 5). Les principaux toxiques dosés en continu sont :

- oxyde de carbone CO
- dioxyde de carbone CO_2
- oxygène O_2
- oxyde d'azote NO - NOx
- hydrocarbures totaux
- chlorures Cl^- et cyanures CN^-

. densité des fumées.

L'étude analytique est complétée par une étude toxicologique sur l'animal :
. toxicité suraiguë alvéolaire sur le lapin ;
. toxicité en ventilation spontanée sur le rat;
. toxicité comportementale sur la souris.

RESULTATS ET DISCUSSION

Les produits de décomposition thermique de trois matériaux (peuplier, chlorure de polyvinyle-PVC, polyuréthane-PU) ont été étudiés selon deux protocoles opératoires:
. décomposition lors d'une montée progressive de température de 20 à 560° C ;
. décomposition à température fixe 450° C.

Afin de pouvoir comparer entre eux les résultats obtenus avec chacun de ces trois matériaux, les concentrations des produits de décomposition thermique sont exprimés en mg de toxique dégagé par 1 gramme d'échantillon et en 1 minute.

4.1. - Décomposition thermique par montée progressive de température

4.1.1. - Conditions opératoires

. Température : 20 à 560° C
. Durée totale de l'essai : 30 minutes
. Débit d'air : 1200 l/h.

Echantillon	Dimensions (mm)	Poids (g)
Peuplier	100 x 20 x 7	4,05
PVC	100 x 20 x 3	9,75
PU	100 x 30 x 10	1,15

L'échantillon est placé sur le chariot porte échantillon situé au centre de la zone de chauffage du "modèle feu". Une fois installé le système de chauffage est programmé et déclenché pour atteindre la température de 560° C en 30 minutes.

4.1.2. - Résultats (figures 1, 2, 3)

Les températures de dégagement maximum des principaux produits de décomposition thermique sont indiqués dans le tableau 1. Les concentrations en toxiques augmentent avec l'élévation de température mais on constate que les températures d'apparition et de dégagement maximum diffèrent suivant le matériau testé. Ainsi, le dégagement maximum d'oxyde de carbone est obtenu pour des températures variant de 350° C pour le peuplier à 515° C pour le PVC.

On peut donc définir pour chaque matériau une "Température critique" qui correspond à la température de dégagement maximum du toxique majeur.

Echantillon	Toxique majeur	Température critique
Peuplier	CO	350° C
PVC	HCl	345° C
PU	HCN	515° C

4.2. - Décomposition thermique à température fixe 450° C

4.2.1. - Conditions opératoires

. Température : 450° C
. Vitesse d'avance du chariot : 1 cm/mn
. Durée totale de l'essai : 30 minutes
. Débit d'air : 1200 l/h.

Echantillon	Dimensions (mm)	Poids (g)
Peuplier	300 x 20 x 7	16
PVC	300 x 20 x 3	27
PU	300 x 20 x 10	3,2

L'échantillon est placé sur le chariot porte échantillon situé dans la zone d'attente du "modèle feu" et est progressivement introduit dans la zone de chauffage dont la température a été stabilisée à 450° C.

4.2.2. - Résultats (figures 4, 5, 6)

Les concentrations en produits de décomposition augmentent avec le temps. On constate pour les trois matériaux testés une cinétique de dégagement semblable comprenant deux phases successives (figure 7).

- 1° phase jusqu'à la 20ème minute correspondant à une augmentation d'abord lente puis rapide des

concentrations en produits de décomposition. Il faut noter dans le cas du peuplier un dégagement de toxiques beaucoup plus rapide qu'avec le PVC et le PU.

- 2° phase de la 20ème à la 30ème minute. C'est un plateau de stabilisation où la concentration en toxiques n'augmente plus. Il se réalise dans l'enceinte du "modèle feu" un équilibre entre la quantité de toxique dégagée par le matériau et celle évacuée par le flux d'air parcourant la chambre de combustion.

D'autre part, la reproductibilité des atmosphères produites dans le "modèle feu" a été testée. Cinq essais de décomposition thermique à 450° C ont été effectués sur chaque matériau. Le tableau (2) décrit un résumé des résultats obtenus et de leurs pourcentages de variations. On peut conclure à une reproductibilité très satisfaisante de ce "modèle feu" puisque l'on obtient des pourcentages de variation d'environ 10 % à l'équilibre. En ce qui concerne les toxiques dosés par potentiométrie (HCl - HCN) le coefficient de variation est plus important car l'affusion de réactif (AgNO3) est fonction d'une différence de potentiel minimale entre les deux électrodes (référence et mesure).

CONCLUSION

On peut conclure du présent exposé que nous disposons d'un "modèle feu" permettant une reproduction satisfaisante d'atmosphères toxiques et la maîtrise de nombreux paramètres pouvant influencer sur leur composition. Les résultats des premières études toxicologiques seront relatées dans une prochaine communication.

TEMPERATURE (° C)	PEUPLIER	PVC	PU
335	Fumées	-	Fumées
345	-	NOx HCl	-
350	Hydrocarbures CO	-	-
390	NOx	-	Hydrocarbures
410	CO_2	-	-
430	-	Fumées	-
460	-	Hydrocarbures	CO
515	-	CO	HCN
550	-	CO_2	NOx

TABLEAU N° 1 : Température de dégagement maximum des principaux produits de décomposition thermique.

Temps (mn)	Echantillon	CO	CO_2	Hydrocarbures	NOx	HCN	HCl
10	Peuplier	110,8 ± 13 %	549,2 ± 13 %	65,7 ± 23 %	0,147 ± 39 %	-	-
	PVC	11,4 ± 25 %	38,2 ± 48 %	58,3 ± 11 %	-	-	238,1 ± 31 %
	PU	66,8 ± 19 %	-	78,5 ± 9 %	2,8 ± 36 %	1,0 ± 36 %	-
20	Peuplier	154,9 ± 11 %	919,6 ± 11 %	80,6 ± 11 %	0,187 ± 30 %	-	-
	PVC	42,1 ± 8 %	351,3 ± 10 %	77,4 ± 5 %	-	-	265,8 ± 31 %
	PU	143,4 ± 19 %	-	110,5 ± 16 %	9,8 ± 11 %	7,5 ± 33 %	-
30	Peuplier	150,8 ± 11 %	1031,9 ± 5 %	79,8 ± 10 %	0,195 ± 29 %	-	-
	PVC	60,8 ± 7 %	554,5 ± 10 %	82,8 ± 11 %	-	-	338,7 ± 25 %
	PU	108,3 ± 9 %	-	125,0 ± 9 %	14,0 ± 4 %	8,0 ± 25 %	-

TABLEAU N° 2 : Résultats à la 10ème, 20ème et 30ème minutes : pourcentages de variation.

FIGURE N° 1

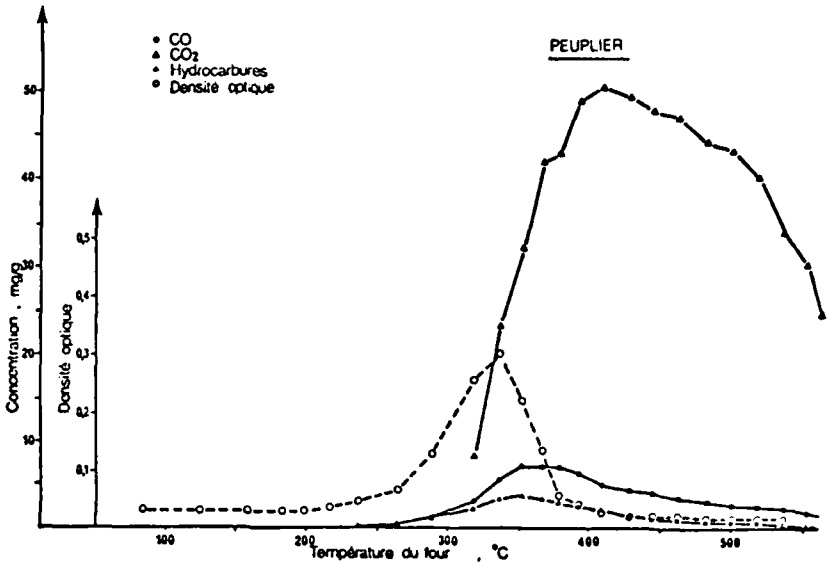


FIGURE N° 2

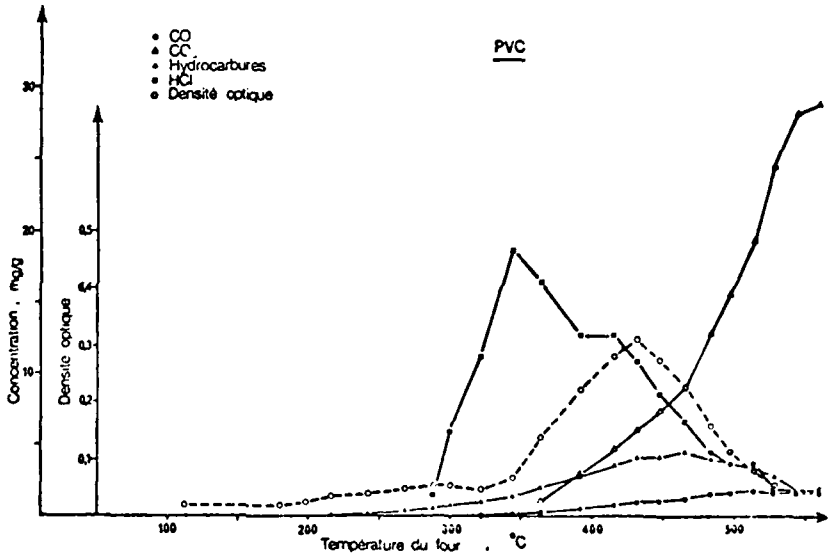


FIGURE N° 3

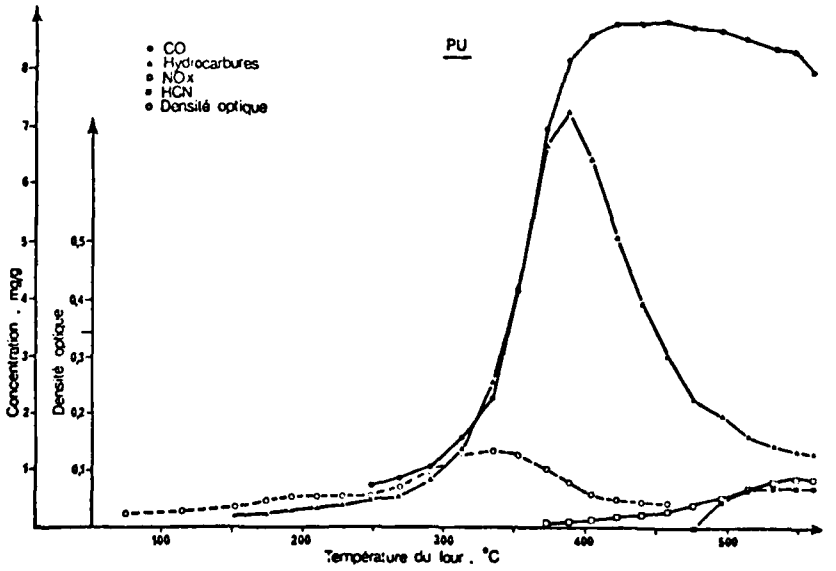


FIGURE N° 4

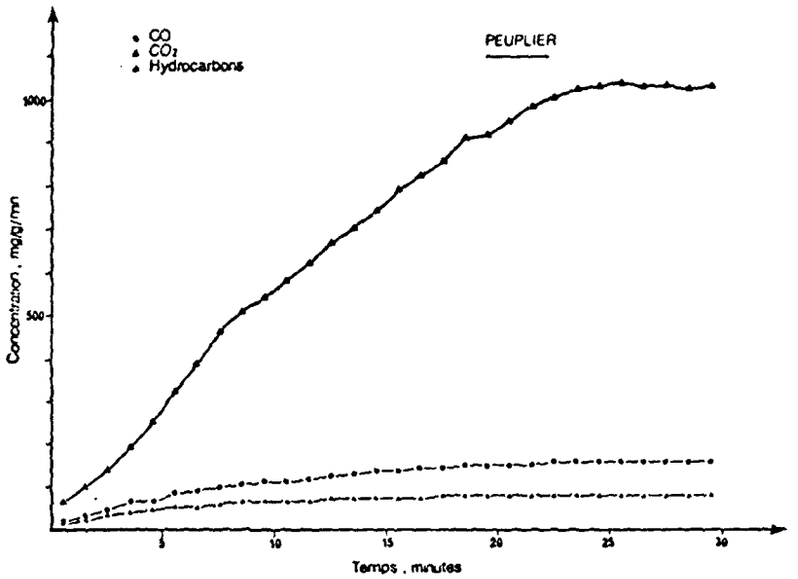


FIGURE N° 5

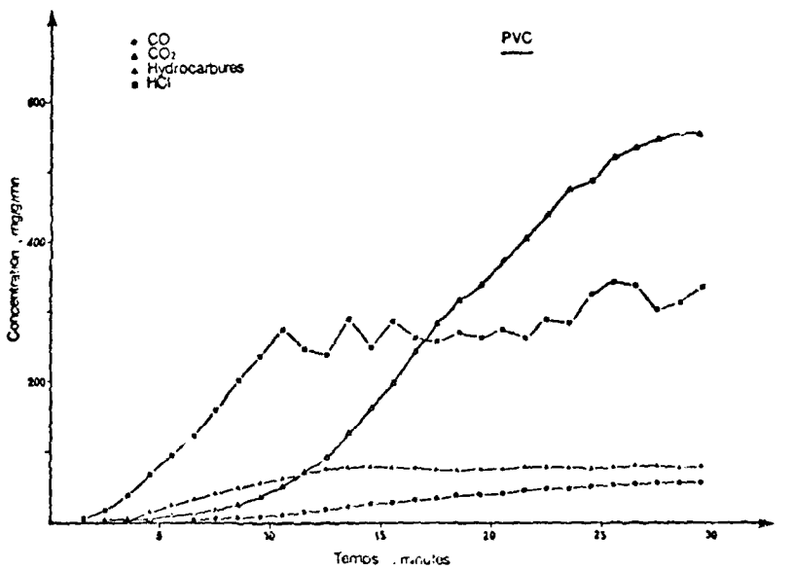
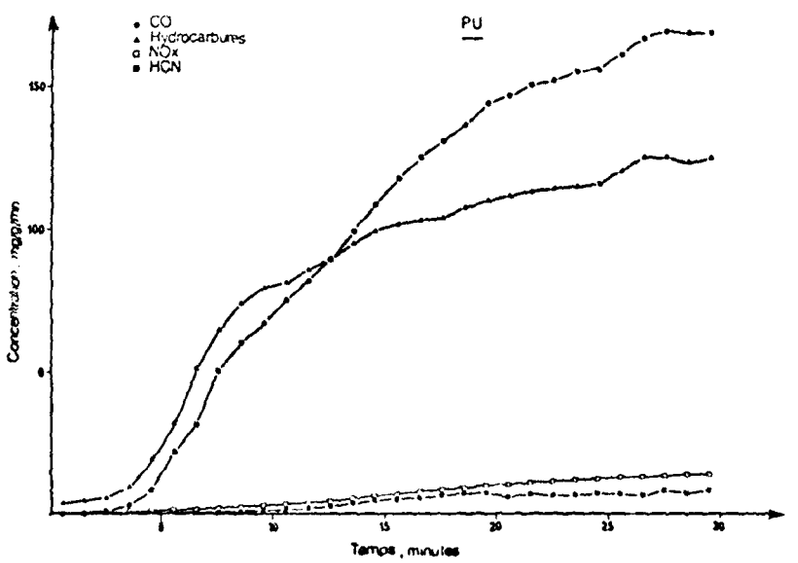


FIGURE N° 6



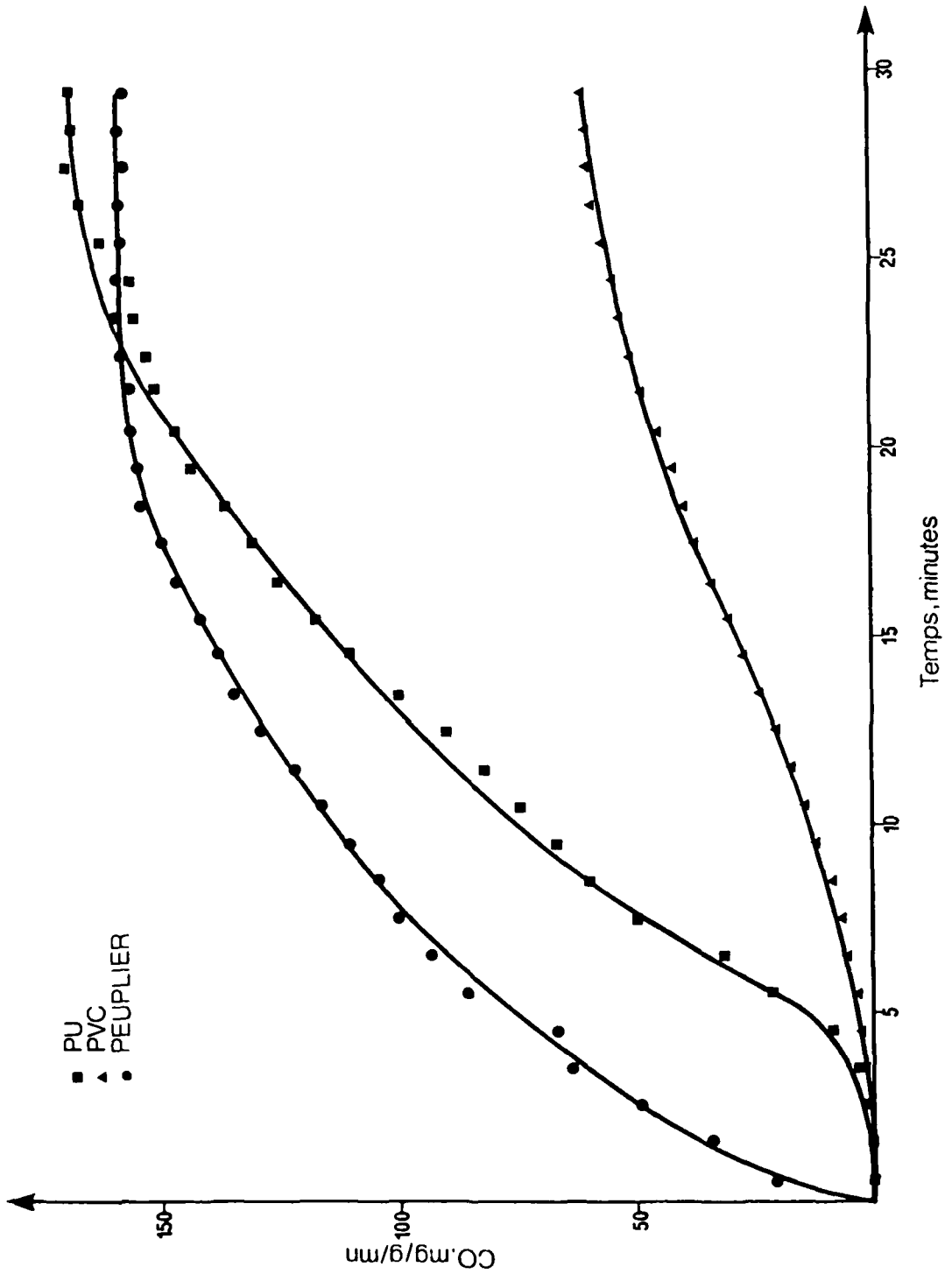

PU
PVC
PEUPLIER
CO.mg/g/mn
150
100
50
5
10
15
20
25
30
Temps,minutes

FIGURE N° 7

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ACUTE CARBON MONOXIDE POISONING

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INTRODUCTION

The principal toxic action of carbon monoxide is accepted as being due to its combination with hemoglobin to form carboxyhemoglobin (COHb). This has the effect of diminishing the oxygen carrying capacity of the blood as well as altering the oxygen dissociation characteristics of the remaining oxyhemoglobin. This fundamental action of carbon monoxide is utilized as an objective measure of the degree of exposure and resulting intoxication based upon the level of COHb produced. The relationship between variable physiological parameters such as diffusivity of the lung, the ventilation rate and the affinity of blood for CO, the CO concentration in inspired air and the exposure time has provided a valuable means of predicting COHb formation (1-4).

STANDARDS FOR ACUTE CO EXPOSURE

The formation of COHb is utilized as the indicator of toxicity in the establishment of standards for maximum CO exposures. These standards are based upon dosages (Ct), determined by the concentration of CO in the ambient air and the time duration of exposure. As these relationships have been well established, the resulting COHb levels may be predicted. Such a relationship is illustrated in Fig. 1 based upon the equation of Coburn et al. (3) applied to short term exposures for individuals engaged in heavy work. Maximum exposure limits have been derived in the formulation of standards such as is illustrated in Figure 2 (5). On the basis of the relationship illustrated in Fig. 1 the standard shown in Fig. 2 would limit COHb formation to 10%.

FIGURE 1

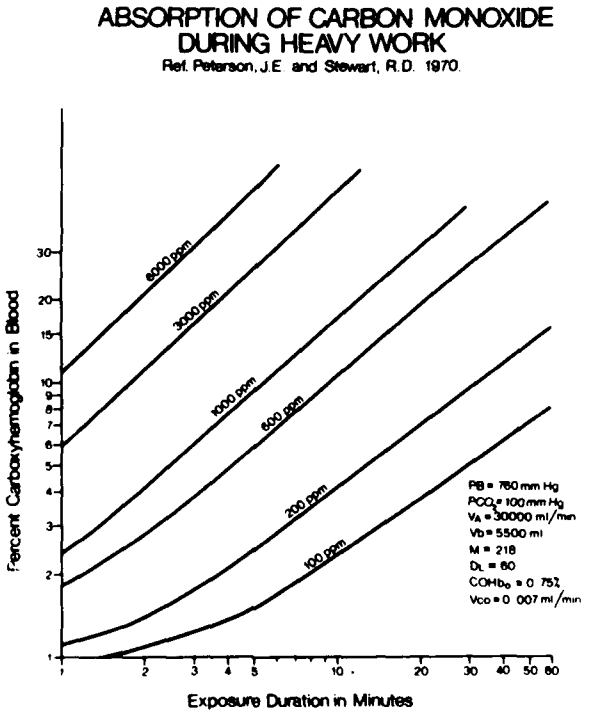
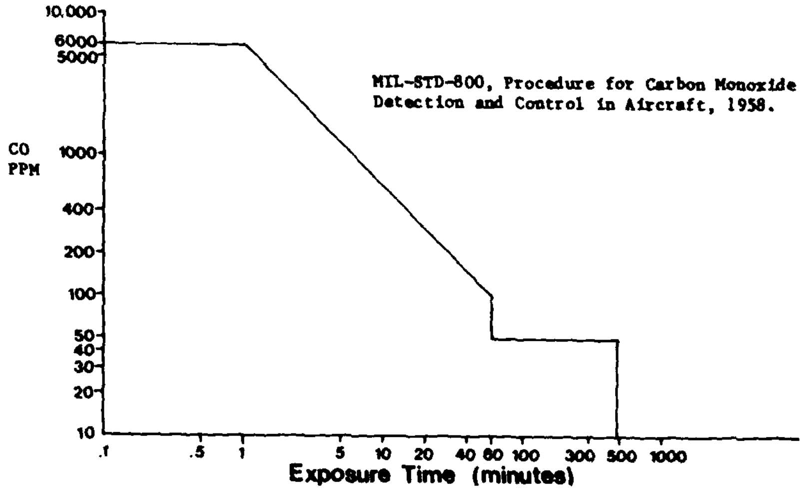


FIGURE 2

Maximum Allowable CO Concentration



This standard provides useful guidelines for maximum exposure dosages, however, there may exist a diminishing margin of safety which it offers in the range of short term, high concentration exposures.

FIELD EXPOSURE EXAMPLES

Diving

A human exposure to a known high concentration of CO was found in a Canadian sports diving accident occurring in 1977. This accident involved seven people. The individuals in this accident had the misfortune of charging their diving tanks from an overheated high pressure air compressor. A post accident analysis of the air in the last tank charged from this compressor is shown in Table I.

TABLE I

CONTAMINATED DIVERS BREATHING AIR ANALYSIS

Oxygen	[illegible] %
Nitrogen	78.1%
Carbon Dioxide	[illegible] PPM
Methane	550 PPM
Ethane	[illegible] PPM
Ethylene	[illegible] PPM
Other Hydrocarbons	
as Methane Equivalent	[illegible] PPM
Water Vapour	[illegible] PPM
Carbon Monoxide	[illegible] PPM*

As divers obtain their entire life support air from that which is [illegible] tanks, these individuals were exposed to air containing a maximum of [illegible] ppm [illegible] duration of their attempted dive. The dive commenced by the individuals walking out from [illegible] line, inserting their mouth pieces and commencing to swim. All of the seven divers [illegible] exposed to the contaminated air, reported a rapid occurrence of dizziness, disorientation and the loss of the sense of direction and depth. The one diver utilizing the air illustrated in Table I was rendered unconscious. The police investigation report indicated that the effects were produced within one to two minutes of commencing the dive. [illegible] the close proximity to the shore and the presence of seven other divers who did not have contaminated air, rescue was initiated immediately and was completed within five minutes. [illegible] air, and was [illegible] Although [illegible] the extreme rapidity of the [illegible]

[illegible]

[illegible]

The profiles for CO evolution within the confines of an armoured fighting vehicle during the test firing of a 7.62 mm and 50 cal machine gun carried out by the authors are illustrated in Figures 3 and 4.

FIGURE 3

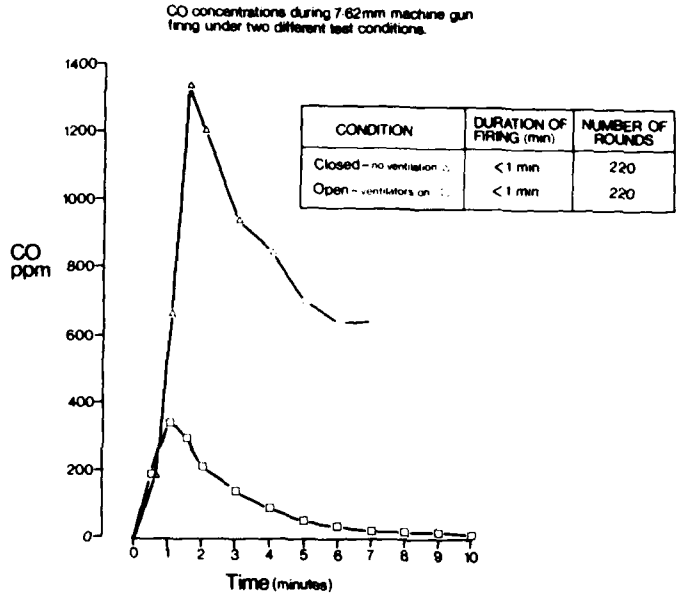
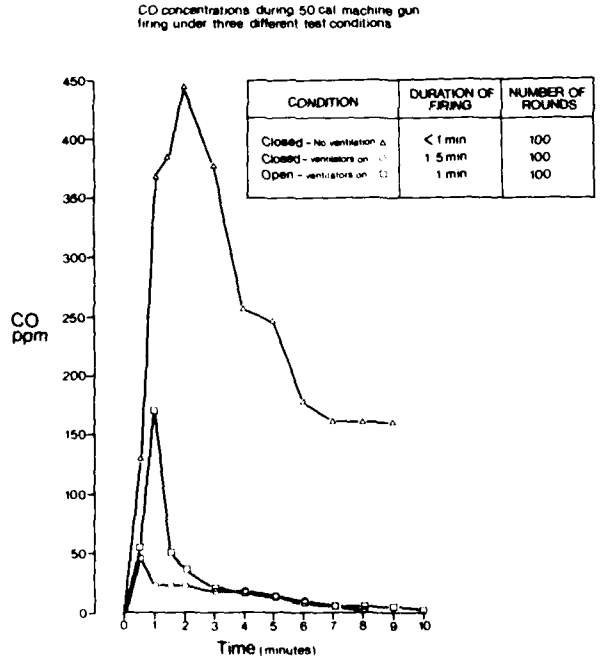


FIGURE 4

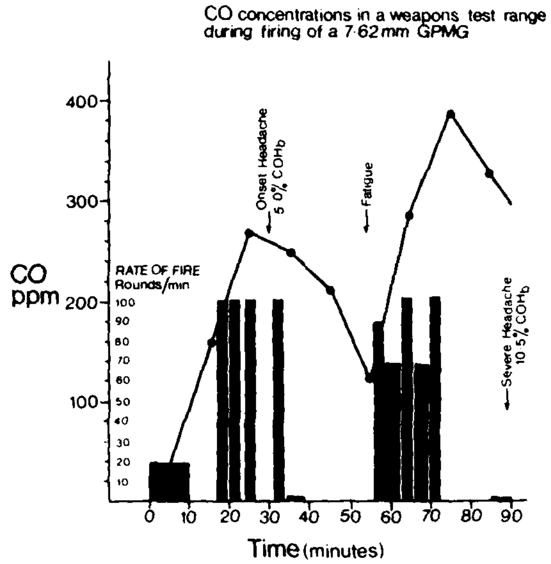


The protocol for the testing of weapons in armoured vehicles under static conditions calls for the procedure to be carried out by means of remote weapons firing and CO sensing if possible. Where test personnel must occupy the interior of the vehicle, a maximum dosage (Ct) of 6000 ppm min is not to be exceeded. This criteria is illustrated in Figure 3 where under the closed down condition with no ventilation, a Ct of 5000 ppm min was achieved in seven minutes and the test was terminated. The COHb of a non-smoking individual exposed to this condition determined by the measurement of CO in expired air (6), was found to be 6.4% at the end of this period.

Occupational Exposure

Very high dosages of CO may be received as the result of more prolonged exposures to lesser concentrations of carbon monoxide. Such a condition is illustrated in a carbon monoxide survey conducted in a poorly ventilated weapons test range building. The CO profile measured during this survey is illustrated in Figure 5.

FIGURE 5



The CO was measured in the immediate vicinity of the weapons technician operating the range. As all firing was conducted by remote methods, behind a protective barrier, his activity was judged to be that between sedentary and light work. The dosages received were 8200 ppm min after 45 minutes of firing and 25000 ppm min after 90 minutes of firing. The weapon technician was a non-smoker and COHb determinations made by the measurement of CO in expired air at these two time periods were found to be 5.0% and 10.6% respectively. This is illustrated in Figure 5. In addition, the sequence of the development of subjective indications of CO poisioning are also shown.

DISCUSSION

The examples of carbon monoxide poisoning presented represent three different intensities of exposure, ranging from high concentration (6000 ppm) for a short exposure period of [illegible] to 5 minutes, a mean concentration of 716 ppm for seven minutes and a mean concentration of [illegible] ppm for 90 minutes. These exposures reinforce the importance of the rate of the challenge upon the resultant toxic effects.

Although the value of utilizing COHb levels as a measure of CO intoxication is not challenged, it still is questioned as to whether COHb formation is the sole cause of the carbon monoxide toxicity. Experiments were conducted by Goldbaum et al. ([illegible], 8) in which the COHb of dogs was elevated [illegible] to [illegible]% by transfusion with COHb saturated blood, as well as i.p. injections of CO to produce animals with the COHb as high as 90%, all without overt toxic effects. These observations raise questions regarding COHb induced anoxia as the principal cause of intoxication. Alternate actions of CO, which involve the intracellular binding to other hemoproteins, have been recently reviewed (9). Other possible mechanisms of toxic action which have been advanced involve CO binding to cytochrome oxidase and myoglobin. Although CO may compete favourably with oxygen for cytochrome oxidase (10), no evidence has yet been presented to indicate that the [illegible] intracellular ratio in the vicinity of the mitochondria is sufficiently low for this to take place. On the other hand it is known that there is a significant binding of CO to myoglobin in the myocardium and skeletal muscles at COHb saturations as low as [illegible]% to [illegible]% ([illegible]), [illegible]; however, the effect of CO bound myoglobin on cellular respiration is only inferred. The high affinity of CO for tryptophan oxidase and catalase has been demonstrated ([illegible]); thus, the possibility of CO inhibition of these enzymes having a role in CO toxicity has also been hypothesized ([illegible]).

Studies both in man and in isolated tissue have presented evidence for CO as a metabolic poison. Non-smokers [illegible] sufficient CO to raise their COHb to only 3.0% ± 1.9% exhibited a [illegible]% increase in mean oxygen debt and related [illegible] increased oxygen consumption during a five minute exercise period on a bicycle ergometer. This increase was not explained by the small reduction in oxygen carrying capacity of the blood by the COHb ([illegible]). An in vitro assessment of the direct cellular toxicity of CO [illegible] electrical activity of cultured cerebellar Purkinje cells of the rat [illegible] depression of activity with a threshold depression occurring at [illegible] ppm and [illegible] depression occurring at 5000 ppm ([illegible]). The depression of cellular activity

produced by CO was shown to be greater than that resulting from hypoxia alone. These authors further demonstrateed that the CO effects were reversed by light indicating a photodissociation of CO from its site of binding as would be the case for an iron-porphyrin complex such as cytochrome a_3 oxidase.

The investigations reviewed point towards carbon monoxide having other possible mechanisms of toxicity in addition to the formation of COHb.

CONCLUSION

Current military standards for maximum CO exposure consider the time-concentration relationship of CO exposure and are based upon the effects known to be produced at varying COHb levels. While the formation of COHb is a principal determinant of CO poisoning it may not reflect the true severity of intoxication for brief exposures to high concentrations. For this reason, as well as the potential for other toxic actions of CO exerting their effect, the application of the upper limits of exposure in standards must be viewed as having a very small margin of tolerance towards the prevention of decrements in performance or the assurance of human safety.

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INFLUENCE DE L'ALTITUDE SUR LA TOXICITE DES OXYDES DE CARBONE

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INTRODUCTION

Dans les conditions normales du vol et essentiellement du fait de la ventilation liée à la pressurisation, l'atmosphère des cabines d'aéronefs ne présente aucun signe de pollution même pour des vols de longue durée (VIEILLEFOND et Col 1977).

Il n'en va pas de même lorsqu'un incendie se déclare à bord ou lorsque par suite d'une avarie du système de climatisation des gaz d'échappements des propulseurs pénètrent dans la cabine.

Des accidents aériens récents ont montré qu'une intoxication massive de l'équipage et des passagers pouvait avoir lieu avant même la destruction de l'avion par le feu ou le crash.

Dans ce genre d'accident, les polluants gazeux libérés dans la cabine peuvent être extrèmement variés, mais les oxydes de carbone sont toujours présents.

La toxicité des oxydes de carbone est aujourd'hui bien connue mais on s'est moins intéressé à une éventuelle potentialisation de leurs effets toxiques par l'altitude.

Le but du travail présenté ici est de préciser le rôle d'une diminution de la pression partielle d'oxygène sur la toxicité d'un mélange inspiré contenant du monoxyde ou du dioxyde de carbone. Les études ont été menées sur l'homme pour le dioxyde de carbone et pour des raisons de sécurité sur l'animal en ce qui concerne le monoxyde.

Dans le premier cas on a cherché à mettre en évidence une éventuelle dégradation de la performance psychomotrice d'un opérateur humain travaillant en altitude, en même temps qu'étaient étudiées les réactions ventilatoires et cardio-vasculaires.

Dans le deuxième cas on a cherché à établir si l'hypoxie interférait sur la concentration léthale 50 p. cent des oxydes de carbone chez l'animal.

METHODOLOGIE ET PROTOCOLE

1 - Chez l'homme l'étude a été menée en trois phases successives.

La première effectuée au sol (PB moyenne = 1007 mb) avait pour but d'acquérir les données témoins en dehors du stress altitude.

La seconde a été effectuée au caisson d'altitude à 2500 m soit PB = 795 mb.
La troisième enfin a été conduite à 5000 m soit PB = 542 mb.

Au cours de chacune des trois phases les sujets ont inhalé un mélange composé soit d'air normal soit de mélanges enrichis en gaz carbonique de façon à atteindre les concentrations de 0,5 - 1 - 2 et 4 p. cent.

Les pressions partielles inspirées en CO_2 varient par conséquent au sol de 2,25 à 30 torr, à 2500 m de 1,8 à 24 torr et à 5000 m de 1,5 à 16 torr. Les mélanges ont été obtenus par une méthode volumétrique et controlés par spectométrie de masse.

Un masque orofacial type aviation permettait l'inhalation du mélange respiratoire et la mesure du débit ventilatoire par pneumotachographie. La saturation en oxygène de l'hémoglobine artérielle a été controlée en continu.

Au plan cardiovasculaire, les techniques de pléthysmographie par impédance électrique ont permis de mesurer les variations du débit aortique et du débit cérébral.

Enfin, la performance psychomotrice des sujets d'expérience a été appréciée en étudiant les résultats obtenus lors d'une tache complexe associant un test de poursuite compensée sur deux axes représentant la tache principale et un test de détection de signaux lumineux aléatoires réalisant la tache secondaire.

Quatre sujets volontaires adultes, en bonne santé, et bien entrainés aux tests psychomoteurs ont participé à l'expérimentation. Le même protocole a été utilisé aussi bien au sol qu'en altitude et pour chacune des concentration en dioxyde de carbone.

Le sujet équipé des électrodes de recueil et des différents capteurs est installé assis devant la batterie de tests de performance. Equipé du masque inhalateur il respire l'air ambiant. Le caisson est alors amené à la pression correspondante à l'altitude choisie. Lorsque celle ci est stabilisée le départ de l'expérience est donné et le sujet commence à inhaler le mélange respiratoire réalisé pour l'essai du jour.

L'enregistrement des paramètres physiologiques est effectué à la cinquième minute.

A la 10 ème minute le sujet effectue un test psychomoteur qui dure lui même 10 minutes.

A l'issue de celui ci un nouvel enregistrement des paramètres physiologiques est effectué.

L'expérience comporte ainsi quatre tests de dix minutes séparés par des temps de repos de 10 minutes dont les cinq premières servent aux enregistrements physiologiques. De telle sorte que la durée totale de l'expérience est de une heure trente.

2 - En ce qui concerne l'expérimentation animale :

L'altitude a été simulée en rétablissant au sol, la pression partielle d'oxygène régnant au sol à 2000 et 5000 m. L'exposition des animaux a été réalisée dans une cage spécialement conçue et permettant la préparation extemporanée des mélanges gazeux enrichis en CO ou CO_2 e en oxygène sans risque pour les manipulateurs.

Grâce à un système de sas, les animaux étaient introduits brusquement dans l'ambiance toxique. le temps d'exposition a été fixé à 15 minutes pour tous les essais. Ce temps parait représentatif des conditions aéronautiques puisqu'en vol il ne peut être question ni d'évacuer un appareil en moins d'un quart d'heure après le début d'un incendie, ni d'accroitre la ventilation, celle ci entrainant une propagation de l'incendie.

La toxicité a été évaluée par la méthode classique des concentrations léthales 50 p. cent. Il a été tenu compte dans le dénombrement des décès de ceux survenus dans les 48 heures qui suivent l'exposition. L'animal choisi était le rat WISTAR femelle pathogen-free de 100 à 120g.

RESULTATS

L'étude de la toxicité aïgue des oxydes de carbone chez l'animal a fourni les résultats suivants.

En ce qui concerne le dioxyde de carbone, la toxicité , exprimée en pression partielle léthale pour 50 p. cent des animaux, est relativement indépendante de l'altitude. Elle passe de 39 kPa au sol à 36 kPa à 2000 et 5000 m pour une exposition de 15 minutes.

Au contraire, la concentration toxique en monoxyde de carbone exprimée de la même façon est très sensiblement proportionnelle à la pression barométrique. En effet elle est de 8,2 hPa au sol mais seulement de 6,15 hPa à 2000 m et de 4,48 hPa à l'altitude de 5000 M.

Ce résultat ne fait que confirmer les données de la littérature concernant le mode d'action toxique de l'oxyde de carbone par fixation peu réversible sur l'hémoglobine avec déplacement de l'oxygène.

L'inhalation en altitude de mélanges hypercapniques apporte chez l'homme des modifications des grandeurs cardiovasculaires et de la performance psychomotrice.

Les résultats de la performance psychomotrice montrent d'une part qu'il existe une variabilité interindividuelle vis à vis des effets du dioxyde de carbone comme de l'altitude et que d'autre part l'effet du gaz carbonique est différent selon l'altitude considérée.

Certains sujets améliorent leur performance en altitude et, lorsque la fraction inspirée de CO_2 augmente à une altitude donnée. D autres au contraire, diminuent leur performance en altitude quelle que soit la valeur de l'hypercapnie. Les derniers enfin diminuent leur performance lorsque la pression partielle de gaz carbonique augmente dans l'air inhalé au sol ou à faible altitude mais l'améliorent lorsqu'elle augmente à 5000 m.

Cependant si l'on fait abstraction de ces différences interindividuelles, on s'aperçoit qu'au sol et à 2000 m la performance reste stable en fonction de la fraction inspirée en gaz carbonique ($F_I CO_2$). Par contre à 5000 m la performance est très dégradée pour $F_I CO_2$ nulle tandis qu'elle revient à des chiffres voisins de la normale pour $F_I CO_2 = 0,04$. Enfin au cours d'une même expérience, on assiste avec le temps à une amélioration de la performance, le dernier test étant significativement amélioré au risque de 1 p. cent par rapport au premier.

Il n'a pas été possible de mettre en évidence un rôle quelconque de $F_I CO_2$ sur le temps de réaction mesuré lors du test d'extinction des signaux lumineux. Par contre l'altitude allonge significativement le temps de réaction.

Les grandeurs physiologiques respiratoires et cardiovasculaires sont classiquement modifiées par l'hypoxie d'altitude et par l'hypercapnie.

Un séjour d'une heure trente à l'altitude de 2000 m n'influe pas sur la ventilation. Ce résultat est en accord avec le travail de GUE et Coll publié en 1974.

Par contre à 5000 m on observe une augmentation de 30 p. cent de la ventilation par rapport au sol. Cette augmentation est due à la fois à l'accroissement du volume courant et de la fréquence respiratoire. Des résultats analogues ont été décrits par PUGH (1964) SOGEL et Col (1967) SALLIN (1968 .

Dans nos expériences, quelle que soit la valeur de la pression barométrique, la ventilation minute s'accroit lorsque la fraction de CO_2 inhalée augmente.

Mais ici l'augmentation du débit est essentiellement liée au volume courant. Cette augmentation est au sol de 37 p. cent lorsqu'on passe de l'air à un mélange de 1 p. cent de CO_2. En altitude, l'augmentation du volume courant en fonction de la fraction inhalée de gaz carbonique est plus faible.

Ainsi à 5000 m elle n'est que de 7 p. cent lorsqu'on passe de l'air à un mélange à 1 p. cent de CO_2. L'augmentation de la fréquence respiratoire est par contre plus importante qu'au sol.

Les grandes variations individuelles dans la réponse ventilatoire au stimulus CO_2 permettent de classer les sujets en deux catégories. Les uns ont une réponse intense et précoce, les autres présentent une faible sensibilité au CO_2.

SHAFFER (1958) LAMBERTSEN (1960) et KELLOG (1964) ont eux aussi décrit des pentes différentes d'un sujet à l'autre, de la courbe de réponse du système ventilatoire au stimulus CO_2. Pour ARKINSTALL et Coll (1974) cette différence de sensibilité est d'origine génétique mais il n'est pas exclu que ceux de nos sujets qui sont très habitués professionnellement aux expérimentations en altitude aient acquit une moindre sensibilité au CO_2, telle qu'on la rencontre chez les sujets vivants en haute altitude.

La saturation en oxygène de l'hémoglobine (SaO_2) subit une chute de 3,5 p. cent après trente minutes de séjour à 2000 m. SIME et Coll (1974) ont rapporté une chute de 3,7 p. cent après quelques heures de séjour à AREQUIPA (ville peruvienne implantée à 2500 m). Nos résultats sont parfaitement concordants.

En plus haute altitude et du fait de la pente de la courbe de Barcroft, la desaturation est évidemment plus importante. Après une heure à 5000 m, la SaO_2 a baissé de 36 p. cent par rapport à sa valeur du niveau de la mer.

La moyenne altitude n'interfère pas sur la valeur du débit cardiaque par contre à 5000 m l'augmentation du débit est plus sensible, de l'ordre de 10 p. cent. En fonction de la $PiCO_2$, il faut attendre des valeurs élevées pour voir s'accroitre le débit cardiaque si l'altitude reste modérée. Mais à 5000 m il croit en fonction de $PiCO_2$.

L'évolution du débit cérébral est tout à fait parallèle à celle du débit cardiaque.

Il est intéressant de noter que l'augmentation du débit cérébral en fonction de $PiCO_2$ n'est franche que jusqu'à la concentration de 2 p. cent. Il semble ensuite y avoir un épuisement du stimulus CO_2. Ce phénomène est probablement lié à la diminution du débit aortique dû à la bradycardie qu'entraine l'hypercapnie.

Cette diminution relative du débit cérébral à 4 p. cent de CO_2 n'est toutefois pas suffisante pour entrainer une dégradation de la performance même en altitude parce que l'augmentation de la ventilation pulmonaire suffit à elle seule à maintenir presque intacte la SaO_2.

En conclusion l'inhalation de mélanges hypercapniques n'entraine jusqu'à 2000 m aucune modification physiologique autre qu'une hyperventilation.

Celle ci est suffisante pour maintenir la saturation du sang artériel en oxygène à un niveau satisfaisant de telle sorte que la performance psychomotrice ne subit aucune dégradation pendant une heure et demi.

En haute altitude (5800 m) l'hypercapnie est, tant qu'elle reste modérée, responsable d'une élévation du débit cardiaque et du débit artériel cérébral. Lorsque l'hypercapnie devient importante la bradycardie qui s'installe entraine une diminution progressive des débits vasculaires. Toutefois la baisse des débits est toujours largement compensée par l'hyperventilation résultant de la synergie du stimulus hypoxique et du stimulus hypercapnique. Si bien que l'on observe pas de chute significative de la performance psychomotrice. Par contre à 5000 m, les concentrations en dioxyde de carbone de 0,5 et 1 p. cent sont incapables de corriger les effets néfastes de l'hypoxie.

L'hypercapnie s'avère ainsi relativement capable de corriger les troubles d'hypoxie d'altitude tant qu'elle ne s'accompagne pas d'une inhalation d'oxyde de carbone.
L'hyperventilation ne ferait alors qu'accroitre l'intoxication oxycarbonée dont la sévérité augmente avec l'altitude.

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IN-FLIGHT OXYGEN GENERATING EQUIPMENT

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SUMMARY

ON-BOARD INFLIGHT OXYGEN GENERATING SYSTEMS PRODUCE HIGH OXYGEN CONCENTRATIONS OF PHYSIOLOGICALLY ADEQUATE OXYGEN FOR THE AIRCREWS. THE QUALITY OF THE OXYGEN IS DEPENDENT ON THE QUALITY OF THE INPUT AIR AND THE PROVISIONS MADE FOR CONTAMINANT CONTROL. THE MOLECULAR SIEVE MATERIAL PROVIDES UP TO 95% OXYGEN WITH CONTAMINANT REMOVAL AND SEPARATION FROM THE OXYGEN PRODUCT GAS MIXTURE. THE CHLORATE CANDLE OXYGEN GENERATOR PRODUCES ALMOST 100% OXYGEN FOR 30 MINUTES AND HAS EFFECTIVE ADSORBERS TO REMOVE CONTAMINANTS FROM THE OXYGEN PRODUCED. THE FLUOMINE SYSTEM PROVIDES UP TO 98% OXYGEN IN THE PRODUCT GAS AND USES ACTIVATED CARBON AND MOLECULAR SIEVE FILTERS FOR CONTAMINANT CONTROL. THE CONCEPT WITH THE GREATEST POTENTIAL IS THE MOLECULAR SIEVE SYSTEM USING A SPECIFIC TYPE OF SIEVE MATERIAL FOR OXYGEN CONCENTRATION AND EFFECTIVE CONTAMINANT CONTROL.

INTRODUCTION

IN-FLIGHT OXYGEN GENERATING EQUIPMENT OFFERS THE AIRCRAFT DESIGNER AND AIRCREWS A LIFE SUPPORT SYSTEM THAT FREES THE AIRCRAFT FROM GROUND BASED OXYGEN RESUPPLY ACTIVITIES EACH TIME THE AIRCRAFT IS PREPARED FOR A MISSION. THESE ACTIVITIES REQUIRE CONSIDERABLE GROUND SERVICING FACILITIES AND MANPOWER. THE ACTIVITIES REQUIRED TO RESUPPLY THE OXYGEN STORES ON-BOARD AN AIRCRAFT CONSIDERABLY EXTEND THE AIRCRAFT TURN AROUND TIME AT FORWARD BASES, AND THUS THE AIRCRAFT MISSIONS ARE LIMITED TO BASES WITH THIS OXYGEN RESUPPLY CAPABILITY. ON-BOARD IN-FLIGHT OXYGEN GENERATING CAPABILITY WOULD ELIMINATE THE REQUIREMENT FOR FACILITIES FOR OXYGEN GENERATION, STORAGE AND SERVICING AT BASES SUPPORTING MISSIONS REQUIRING FAST TURN AROUND TIME. THIS CAPABILITY WOULD ALSO EXTEND MISSION TIMES BEYOND THOSE WITH THE CONVENTIONAL OXYGEN SYSTEM.

THE CONCEPT OF ON-BOARD GENERATION OF OXYGEN IS NOT A RECENT UNDERTAKING. (1) THE SUBMARINE HAS UTILIZED ON-BOARD OXYGEN GENERATION. THE COAL MINER HAD AN OXYGEN GENERATION KIT TO PROVIDE BREATHABLE OXYGEN IN HAZARDOUS OR TOXIC ATMOSPHERES. THE SPACE PROGRAM WITH THE REQUIREMENT FOR CLOSED ECOLOGICAL SYSTEMS PROVIDED SIGNIFICANT DEVELOPMENT FOR ON-BOARD OXYGEN GENERATION IN SPACECRAFT. FOR AIRCRAFT USE, THE OPEN LOOP SYSTEMS WERE BETTER SUITED WHERE THE REUSE OF THE CLOSED CABIN ATMOSPHERE GASES WAS NOT INTENDED. THE OXYGEN WAS TO BE SEPARATED AND CONCENTRATED USING THE ATMOSPHERE OUTSIDE THE AIRCRAFT. OR, THE OXYGEN COULD BE PRODUCED FROM CHEMICALS STORED ON-BOARD THE AIRCRAFT. THE CONVENTIONAL AIRCRAFT OXYGEN SYSTEM CAPABILITY WAS THUS SPARED OR EXTENDED.

LIMITED QUANTITY OXYGEN GENERATOR

OXYGEN GENERATING EQUIPMENT THAT HAS BEEN DESIGNED FOR AIRCRAFT USE HAVE RANGED FROM A SIMPLE CHEMICAL REACTION DEVICE TO SYSTEMS THAT ARE COMPLEX, HIGH POWER CONSUMING AND EXPENSIVE TO FABRICATE. THE SIMPLE SODIUM CHLORATE OXYGEN GENERATOR HAS LONG BEEN USED BY THE NAVY FOR BREATHING OXYGEN IN SUBMARINES. THIS OXYGEN GENERATOR IN FIGURE 1 HAS BEEN IMPROVED AND IS NOW USED ON AIRLINES AS THE EMERGENCY OXYGEN IN CASE OF CABIN PRESSURE LOSS. WHEN THE HOUSING CAP IS REMOVED AND THE OXYGEN MASK PULLED OUT, A LANYARD FIRES A SQUIB WHICH INITIATES THE REACTION. OXYGEN IS PRODUCED AND IS DUCTED TO THE OXYGEN MASK. FROM THE INITIAL REACTION, THE PRODUCTION OF SOME SALT FUMES, CHLORINE GAS, CARBON MONOXIDE AND CARBON DIOXIDE IS POSSIBLE BUT THE UNITS WERE PROVIDED WITH EFFECTIVE FILTERS SO THAT 100% OXYGEN WAS AVAILABLE. NO REAL TOXIC HAZARD EXISTS WITH THE USE OF CHLORATE CANDLES.

THERE WAS A PERIOD OF TIME WHEN DEVELOPMENTAL EFFORTS STRESSED CLOSED LOOP ECOLOGICAL SYSTEMS WITH BREATHABLE ATMOSPHERES DESIGNED TO USE SUPEROXIDES OF METALS, FOR EXAMPLE POTASSIUM SUPEROXIDE. THIS MATERIAL WAS EFFECTIVE FOR GENERATING OXYGEN AT HUMID CONDITIONS WITH THE ABSORPTION OF CARBON DIOXIDE. PROBLEMS WITH THE SUPEROXIDE MASS OCCURRED WITH CAKING AND REACTION PRODUCT COATING OF THE SUPEROXIDE GRANULES. EXCESSIVE WETTING DETRACTED FROM ITS CONTINUED USE IN MANNED SYSTEMS. HAZARDS OF HIGHLY IRRITATING ALKALINE DUST WOULD BE PRESENT IF THE FILTER FAILED OR THE CONTAINER WAS RUPTURED.

THE USE OF OTHER METAL OXIDES SUCH AS BARIUM OXIDE HEATED TO HIGH TEMPERATURES TO DECOMPOSE THE OXIDE AND RELEASE OXYGEN WAS NOT CONSIDERED PRACTICAL FOR ON-BOARD OXYGEN GENERATION.

CONTINUOUS OXYGEN PRODUCTION BY FLUOMINE

ANOTHER CHEMICAL SYSTEM FOR OXYGEN GENERATION USED A COBALT CHELATE, CALLED FLUOMINE.(2) THIS OXYGEN GENERATION SYSTEM IN FIGURE 2 WAS DESIGNED TO PRODUCE 4.6 LBS/HR OF OXYGEN AND HAD A WEIGHT OF 135 LBS. THE SYSTEM WAS OPERATIONALLY TESTED IN THE C-130 AND THE B-1 AND FOUND TO BE ADEQUATE TO PRODUCE 98 PLUS PERCENT OXYGEN.(3) THE FLUOMINE SYSTEM IN FIGURE 3 UTILIZED TWO CANISTERS OF THE COBALT CHELATE, ALTERNATELY PRESSURIZED WITH CONDITIONED ENGINE BLEED AIR. EACH PRESSURIZED CANISTER WAS THEN DEPRESSURIZED, HEATED

TO 240°F AND EVACUATED TO 3 TO 8 PSIA. A 4-STAGE VACUUM-COMPRESSOR WAS SPECIALLY DESIGNED TO EVACUATE THE CANISTER AND THEN COMPRESS THE EVACUATED OXYGEN TO A PRESSURE OF 1800 PSIA, IN THE HIGH PRESSURE GASEOUS OXYGEN TANK IN THE AIRCRAFT. THE TEMPERATURES ARE RATHER HIGH AND THE OXYGEN GENERATOR REQUIRES A TEMPERATURE CONTROL SYSTEM INTERFACED WITH THE AIRCRAFT TEMPERATURE CONTROL SYSTEM. A HIGH TEMPERATURE OF 210 TO 240°F WITH VACUMM DESORBS THE OXYGEN. THE ABSORPTION OF OXYGEN ONTO THE FLUOMINE IS EFFECTED AT 100°F AND 40 PSIA. OXYGEN IS THUS PROVIDED TO THE AIRCREW THROUGH THE AIRCRAFT GASEOUS OXYGEN TANK AND NO BASIC ALTERATION TO THE AIRCRAFT OXYGEN SYSTEM IS REQUIRED. AN ON-OFF SWITCH IS REQUIRED TO TURN THE SYSTEM ON. THE TEMPERATURE CONTROL, VALVING AND COMPRESSOR ARE AUTOMATICALLY CONTROLLED. WHEN THE PRESSURE IN THE OXYGEN TANK DECREASES WITH AIRCREW USE TO ABOUT 1450 PSIA, THE OXYGEN GENERATION SYSTEM IS ACTIVATED AND OXYGEN IS PRODUCED AND PRESSURIZED INTO THE AIRCRAFT OXYGEN TANK UNTIL A 1800 PSI PRESSURE IS ATTAINED. THE GENERATOR IS THEN AUTOMATICALLY TURNED OFF.

THE FLUOMINE SYSTEM HAS BEEN SHOWN TO BE EFFECTIVE AND ADEQUATE FOR THE DESIGN MISSIONS, BUT IT HAS SEVERAL DISADVANTAGES. THESE ARE THE HIGH COST OF THE COBALT CHELATE, THE RATHER FREQUENT REPLACEMENT OF THE ACTIVE MATERIAL, AND ITS VERY READY INACTIVATION BY MOISTURE WHICH TENDS TO ACCUMULATE ON THE FLUOMINE WITH TIME USE.

WITH ADEQUATE FILTERS AND ADSORBERS, THE TOXIC HAZARDS ASSOCIATED WITH THIS SYSTEM HAVE BEEN NIL. IN DUST FORM THE COBALT CHELATE IS A PRIMARY IRRITANT.

THE FLUOMINE OXYGEN GENERATION SYSTEM IS BEING ACTIVELY SUPPORTED FOR THE B-1 AIRCRAFT USE.

OXYGEN CONCENTRATION ON MOLECULAR SIEVE

ANOTHER ON-BOARD OXYGEN GENERATION SYSTEM (OBOGS) WAS DESIGNED ABOUT THE USE OF MOLECULAR SIEVE (MS), A CRYSTALLINE ALUMINOSILICATE COMPOUND ALSO KNOWN AS ZEOLITE. THE CONCEPT IN FIGURE 4 IS BASED ON THE PHYSICAL SEPARATION OF THE GASES BY DIFFERENTIAL ADSORPTION. THE AIRCRAFT ENGINE BLEED AIR IS COMPRESSED ALTERNATELY ONTO TWO MOLECULAR SIEVE BEDS.(4) THE NITROGEN UNDER PRESSURE IS ADSORBED ONTO THE MOLECULAR SIEVE BY VAN DER WALLS FORCES AND IS RELEASED FROM THE MOLECULAR SIEVE UPON THE RELEASE OF THE PRESSURE. NO CHEMICAL BONDING OF THE OXYGEN TO THE MOLECULAR SIEVE OCCURS SUCH THAT OXYGEN IS CONCENTRATED BY PASSAGE THROUGH THE MOLECULAR SIEVE. NO HEATING OR COOLING IS REQUIRED IN THE OXYGEN SEPARATION. THE RESULT IS A PRODUCT GAS MIXTURE WHICH IS APPROXIMATELY 95% OXYGEN.

PROTOTYPE UNITS OF AN ON-BOARD OXYGEN GENERATION SYSTEM WERE DELIVERED FOR INSTALLATION INTO THE AV-8A (HARRIER) AIRCRAFT. IN FIGURE 5, THE OBOGS WAS COMPARED WITH LIQUID OXYGEN CONVERTERS. THE MOLECULAR SIEVE OXYGEN GENERATION SYSTEM HAD A VOLUME OF 0.88 CU FT AND IT WEIGHED 35 LBS. A 5-LITER LOX CONVERTER HAS A VOLUME OF 1.05 CU FT AND AN EMPTY WEIGHT OF 10.5 LBS APPROXIMATELY AND FILLED WEIGHT OF 25 LBS APPROXIMATELY. CAPACITY IS ABOUT 12.65 LBS OF OXYGEN.

A 10 LITER LOX CONVERTER HAS A VOLUME OF 1.6 CU FT AND AN EMPTY WEIGHT OF 16.25 LBS AND A FULL WEIGHT OF 36 LBS.

RESOURCE REQUIREMENTS FOR THE MS OBOGS UNIT CONSIST OF COMPRESSED AIR SUPPLIED AT 55 LBS PER HOUR AT 8-60 PSIA. THE REQUIRED DC POWER IS 50 WATTS WITHOUT REQUIRED HEATING BUT 600 WATTS WITH HEATING AT EXPOSURES TO LOW TEMPERATURES DOWN TO -65°F. IN FIGURE 6 THE MOLECULAR SIEVE OXYGEN GENERATOR IS SHOWN WITH THE COVER OPENED, THE LOW PRESSURE REGULATOR AND MASK, AND THE OXYGEN SENSOR.

THE CONTRACTOR FOR THE US NAVY OBOGS UNITS PRESENTED SOME MOST FAVORABLE INFORMATION ON THE RELIABILITY AND MAINTAINABILITY. FOR RELIABILITY, A MEAN TIME BETWEEN FAILURE (MTBF) OF 6200 HOURS WAS PREDICTED. THE ONLY MOVING PART WAS A ROTARY VALVE WHICH CYCLED THE PRESSURIZATION AND DEPRESSURIZATION OF THE CANISTERS ON A TIME CYCLE FOR OPTIMUM OUTPUT OF OXYGEN CONCENTRATION AND FLOW. MAINTENANCE PLANNING WAS FOR A 2000 HRS INTERVAL TO REPLACE THE VALVE, MOTOR, AND THE FILTER ELEMENTS. CLEANING, TESTING AND REPLACING THE MODULES WOULD BE PERFORMED WHEN NECESSARY. SINCE THERE WAS LIMITED OPERATION TEST AND EVALUATION DATA ON AN OBOGS ON AN AIRCRAFT, THE MAINTENANCE AND RELIABILITY INFORMATION OF THE CONCEPT IS PRELIMINARY IN NATURE. THE MOLECULAR SIEVE MATERIAL SHOULD BE EFFECTIVE INDEFINITELY, DEPENDING UPON THE QUALITY OF THE INPUT COMPRESSED BLEED AIR AND THE FILTERS. THE INPUT AIR MUST BE DRY AND FREE OF PARTICULATES AND GASEOUS CONTAMINANTS.

THE BLEED AIR FROM AN AIRCRAFT ENGINE IS COMPRESSED, COOLED AND THEN REDUCED IN PRESSURE SO THAT THE AIR AVAILABLE IS DRY AND COOL AND REQUIRES NO REAL CONDITIONING WHEN PROVIDED TO THE MOLECULAR SIEVE BEDS. HOWEVER, THE UNITS AS DESIGNED DO HAVE FILTERS AND DRYING AGENTS.

COST AND ESTIMATES COMPARISONS OF ON-BOARD OXYGEN GENERATION CONCEPTS HAVE ALL BEEN BASED UPON THE COST OF THE LIQUID OXYGEN SYSTEM IN AIRCRAFT AND THE GROUND SUPPORT EQUIPMENT REQUIRED TO GENERATE AND DISTRIBUTE LIQUID OXYGEN AND SERVICE AIRCRAFT. THE INITIAL COSTS OF LOX SYSTEMS WAS BASED ON A 1967 REPORT WHICH WAS ACCOMPLISHED IN EVALUATING AN ELECTRO-CHEMICAL CONCEPT FOR ON-BOARD OXYGEN GENERATION.(5) THE FIGURES HAVE BEEN QUOTED BY CONTRACTORS AND DOD PROJECT ENGINEERS IN COMPARING THE CONCEPTS AND DERIVING COST ESTIMATES OF CURRENT SYSTEMS. THESE NEED TO BE ADJUSTED AND UPDATED FOR INFLATION AND INCREASES IN COSTS OF FUEL AND OF MATERIALS.

FOR A MORE RECENT COMPARATIVE BASIS, USING 300 UNITS AS A BASIS FOR COMPARISONS, THE MOLECULAR SIEVE CONCEPT SHOULD PROVIDE A COST SAVINGS OF APPROXIMATELY 3 TO 1. THE LOX GENERATORS WERE NOT INCLUDED IN THE COSTING COMPARISONS. SOME ITEMS IN THE COMPARISONS ARE PRESENTED IN FIGURE 7.

	LOX		OBOGS	
AIRCRAFT - EQUIPMENT		$ 450,000		$1,860,000
SUPPORT EQUIPMENT		75,000		6,000
LOX PURCHASED		864,000		
LABOR		8,868,000		376,000
DEPOT REPAIR UNITS		1,500,000		1,460,000
SUPPORT EQUIPMENT		75,000		
TOTALS		$11,832,000		3,702,000

THE SAVINGS BASED ON THESE FIGURES WERE $8,130,000. FUEL COSTS IN LOX GENERATION, TRANSPORTATION AND HANDLING ARE A SIGNIFICANT FACTOR. LABOR COSTS ARE ALSO SIGNIFICANT. THESE FIGURES REQUIRE CONTINUED ADJUSTING FOR THE RISE IN FUEL COSTS.

THE COST ESTIMATES OF THE MOLECULAR SIEVE OBOGS UNITS WERE BASED ON R&D UNITS. FROM THE SYSTEM COST FIGURES, AN MS OBOGS UNIT COST WAS ESTIMATED AT $3,200 EACH FOR 300 UNITS. THE R&D UNITS WERE ESTIMATED AT $15,000 EACH IN THE 1978-1979 TIME FRAME. WHEN THE UNIT COST IS EXAMINED, THE HIGHEST COST ITEM COMPONENT IS THE MOTORIZED VALVE. THE MOLECULAR SIEVE MATERIAL (APPROXIMATELY 22 LBS) CAN BE READILY OBTAINED COMMERCIALLY AND IS NOT A HIGH COST ITEM. THE CANISTERS, PIPING, CHECK VALVES AND ORIFICES ARE NOT HIGH COST ITEMS. THE AIRCRAFT MODIFICATION TO PROVIDE THE ENGINE BLEED AIR, THE FILTER UNITS, THE DC POWER, THE OXYGEN MONITOR CAPABILITY, THE VENTING OF THE UNITS ARE ADDITIONAL AND SIGNIFICANT COSTS ASSOCIATED WITH OPERATIONAL DEPLOYMENT.(6) THE OPERATIONAL TEST AND EVALUATION IS NOW BEING CONDUCTED BY THE NAVY ON THE AV-8A. THE AIR FORCE HAS NOT ACTIVELY SUPPORTED THE MOLECULAR SIEVE OBOGS WITH MONEY OR AIRCRAFT. THE ARMY HAS EVALUATED ON-BOARD OXYGEN GENERATION UNITS ON THE OV-1 AND RU-21 AND HAS FOUND FAVORABLE RESULTS. THE PROPOSALS FOR RETROFITTING AF AIRCRAFT HAVE NOT FOUND ADEQUATE SUPPORT FOR OT&E. THE TRAINERS HAVE BEEN PROPOSED AS LIKELY CANDIDATES.

THE MOLECULAR SIEVE OBOGS UNIT WAS DESIGNED TO BE INSTALLED IN THE AIRCRAFT IN THE LIQUID OXYGEN CONVERTER COMPARTMENT. THE OBOGS UNIT REQUIRES A BLEED AIR FEED FROM THE 8TH STAGE BLEED AIR TAP WHICH IS THE ECS AIR SUPPLY. THIS AIR IS COOLED AND DRIED IN AN AIR CYCLE MACHINE TO PROVIDE THE INPUT AIR TO THE OBOGS. THIS INPUT AIR MUST BE AT LEAST 10 PSIG, WITH 30 PSIG AS THE DESIGN PRESSURE. THE MASS FLOW MUST BE 60 LBS/HR. THE OUTPUT OR PRODUCT GAS FROM ONE OBOGS PROTOTYPE WAS 2 TO 2.4 LBS/HR OF 95% OXYGEN. THIS OUTPUT OR PRODUCT GAS IS PIPED UNDER PRESSURE TO A 4-LITER PLENUM AND THEN DIRECTLY THROUGH A LOW PRESSURE REGULATOR TO THE MASK. AN OXYGEN CONCENTRATION MONITOR MUST BE IN THE PRODUCT GAS LINE TO VERIFY THE OXYGEN CONTENT, THUS MONITORING THE OBOGS OPERATIONAL EFFECTIVENESS. THE OUTPUT OR PRODUCT GAS IS 95% OXYGEN; THE REMAINDER IS ARGON AT 5% CONCENTRATION, A CONTAMINANT VERY SIMILAR TO NITROGEN WITH RESPECT TO THE PHYSIOLOGICAL ASPECTS OF A BREATHABLE GAS MIXTURE.

THE PILOT OR CREWMAN WILL SEE A CONTINUOUS FLOW OF OXYGEN ENRICHED PRODUCT MIXTURE PLUS ARGON AT A PRESSURE DEPENDENT UPON THE INLET PRESSURE. FROM USAF SCHOOL OF AEROSPACE MEDICINE DATA AT CABIN ALTITUDES ABOVE 9.1 KM (30,000 FT), THE OXYGEN CONCENTRATION REMAINED IN THE RANGE OF 94-95% AT ALL FLOWS UP TO 78.6 LPM.(7)

GENERALLY AT ALTITUDE, AT THE LOWER INPUT PRESSURE AND AT HIGHER PRODUCT GAS FLOWS, THE OXYGEN CONCENTRATION WAS LOWER. AT THE HIGH INPUT GAS PRESSURES AND AT THE HIGH AIRCRAFT ALTITUDES (CABIN ALTITUDES), A HIGH PERCENT OXYGEN OCCURS IN THE LOW FLOWS OF PRODUCT GAS. WHEN THE CABIN ALTITUDE APPROACHES THE EXHAUST ALTITUDE, THE PERCENT OXYGEN APPROACHES 95%.(8)

TABLE I FROM HUMAN COMPATIBILITY TESTING AT SCHOOL OF AEROSPACE MEDICINE GAVE THE FOLLOWING ATTAINABLE PRODUCT GAS FLOW RATES. (7) (GRAPH 8) LOW PRESSURE OF SUPPLY AIR RESULTED IN LOW PRODUCT GAS FLOW RATE; WITH HIGHER INPUT AIR PRESSURES AT ALTITUDE, HIGHER PRODUCT GAS FLOWS WERE OBTAINED.

TESTING AT THE SCHOOL OF AVIATION MEDICINE INDICATED THAT THEIR PROTOTYPE UNIT PROVIDED ADEQUATE OXYGEN UNDER ALL SIMULATED ANTICIPATED FLIGHT CONDITIONS UP TO AN ALTITUDE OF 28,000 FT AND WITH IMPROVED PRESSURE DEMAND REGULATION WILL PROVIDE ADEQUATE AND SAFE OXYGEN PRESSURE FOR PROTECTION UP TO 44,000 FT. (8)

MOST RECENTLY, FLIGHT TESTING OF THE MS OBOGS UNITS WAS INITIATED AT THE NAVAL AIR TEST CENTER, PATUXENT RIVER, MD ON THE AV-8A HARRIER. THE FLIGHT TESTING FOR TECHNICAL EVALUATION IN EARLY 1980 IS TO BE FOLLOWED BY OPERATIONAL EVALUATION SCHEDULED IN LATE 1980. UPON COMPLETION OF THE TECHEVAL/OPEVAL, UNITS FOR THE AV-8A HARRIER WILL BE ELIGIBLE FOR APPROVAL FOR SERVICE USE, ASD. (9)

THE NAVY EFFORT HAS INVOLVED THE NAVAL AIR TEST CENTER, NAVAL MISSILE TEST CENTER, AND NAVAL AEROSPACE MEDICAL RESEARCH INSTITUTE.

BENDIX, ONE OF THE CONTRACTORS FOR THE MS OBOGS HAS COMPILED SOME 20,000 HOURS OF LABORATORY TESTING. THERE HAS BEEN SOME 75 HOURS OF SUCCESSFUL FLIGHT TEST ON US ARMY OV-1 AND RU-21 BEECH KING AIRE AIRCRAFT AND 33 HOURS ON THE EA-6B PROWLER NAVY AIRCRAFT. THE NAVY PROGRAM WAS PRESENTED WITH THE FOLLOWING GUIDELINES, DATES AND MILESTONES:

MANRATING WAS COMPLETED - 1977, AT USAFSAM
PRELIMINARY FLIGHT EST IN EA-6B PROWLER WAS COMPLETED IN 1979
TECHNICAL EVALUATION WAS PLANNED FOR 1979/1980
HARRIER AV-8A RETROFIT WAS PLANNED IN THE TIME FRAME 1981-1982
ALL CARRIER LOX WAS PLANNED TO BE ELIMINATED.

THE PROBLEMS ASSOCIATED WITH THE MS OBOGS PERTAIN TO THE LOSS OF OXYGEN PRODUCTION WHEN THE INLET AIR PRESSURE IS REDUCED AND THE MASS FLOW IS INADEQUATE. WHEN THE ENGINE BLEED AIR PRODUCTION DROPS DUE TO ENGINE IDLE, ENGINE AIRCRAFT LETDOWN OR ENGINE FAILURE, THE OBOGS PRODUCT GAS FLOW IS REDUCED AND A BACKUP SOURCE OF OXYGEN IS REQUIRED. THIS IS AN EMERGENCY SITUATION AND REQUIRES AN OXYGEN RESERVE BACKUP, WHICH IS A DESIGN REQUIREMENT OF THE MOLECULAR SIEVE OXYGEN GENERATION SYSTEM.

THE CONTAMINATION OF THE MOLECULAR SIEVE WITH WATER REDUCES ITS OXYGEN SEPARATION CAPABILITY SOMEWHAT. THE ENGINE BLEED AIR SUPPLIED TO THE OBOGS MUST HAVE NOT MORE THAN 22 GRAINS OF WATER PER LB OF DRY AIR. THE OTHER MAJOR CONTAMINANTS IN BLEED AIR SUCH AS CARBON DIOXIDE, CARBON MONOXIDE, ALCOHOL, FUEL, AND HYDROCARBON BREAKDOWN PRODUCTS MUST BE AT LOW CONCENTRATIONS TO AVOID CARRY OVER INTO THE PRODUCT OXYGEN.

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CHEMICAL OXYGEN GENERATOR

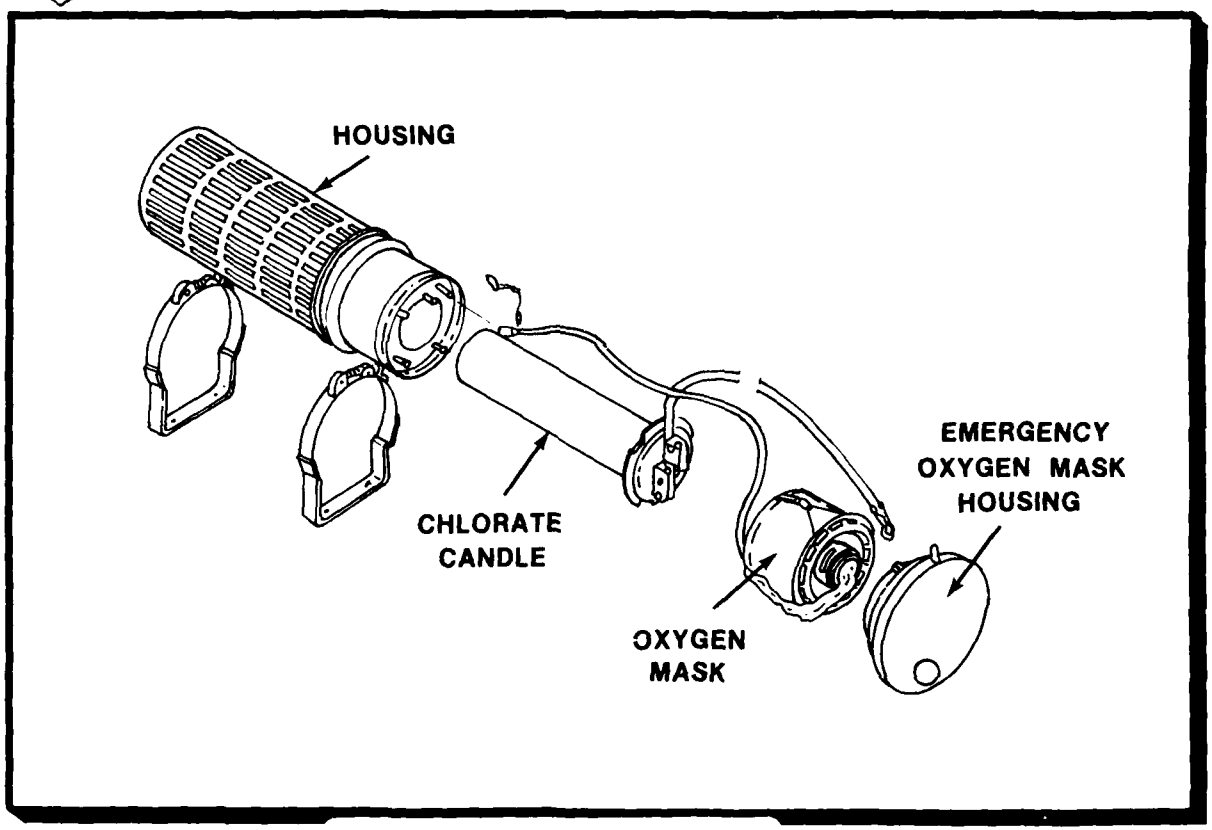

FIGURE 1 - CHEMICAL OXYGEN GENERATOR

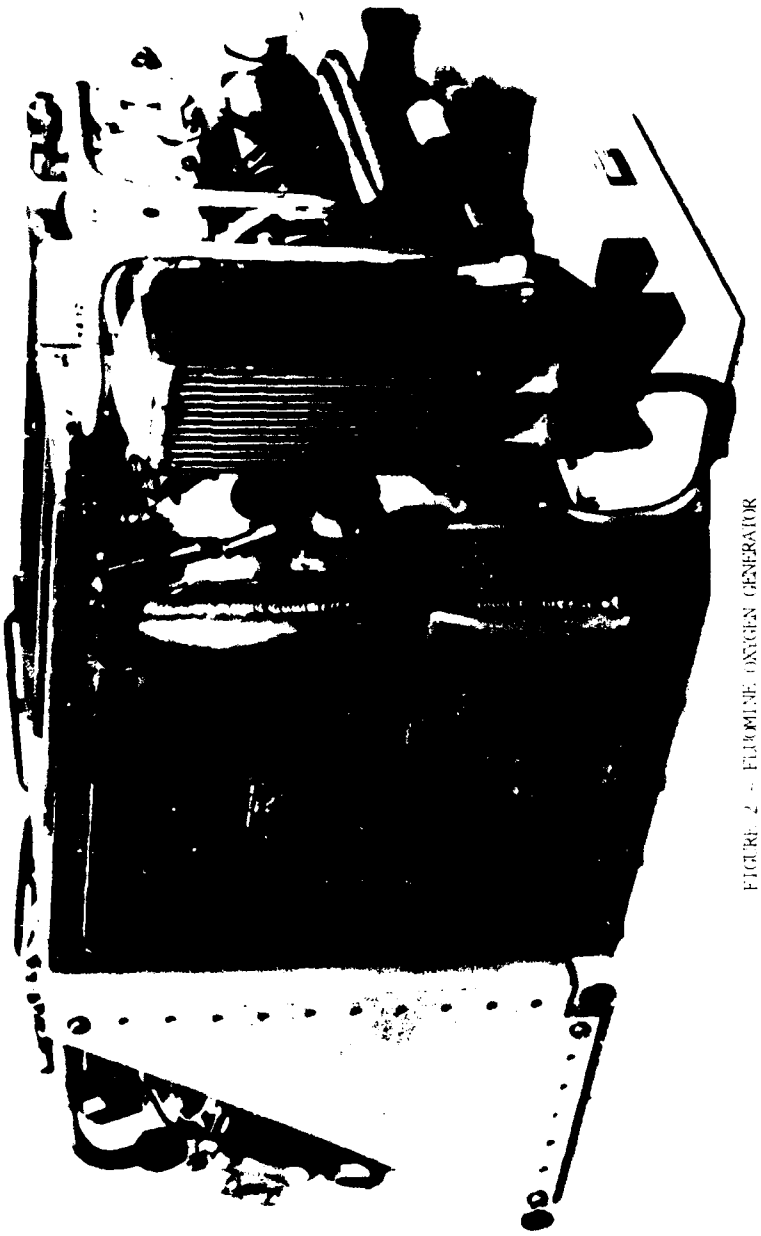

FIGURE 2 – FLUOMINE OXYGEN GENERATOR

OXYGEN CONCENTRATION SYSTEM

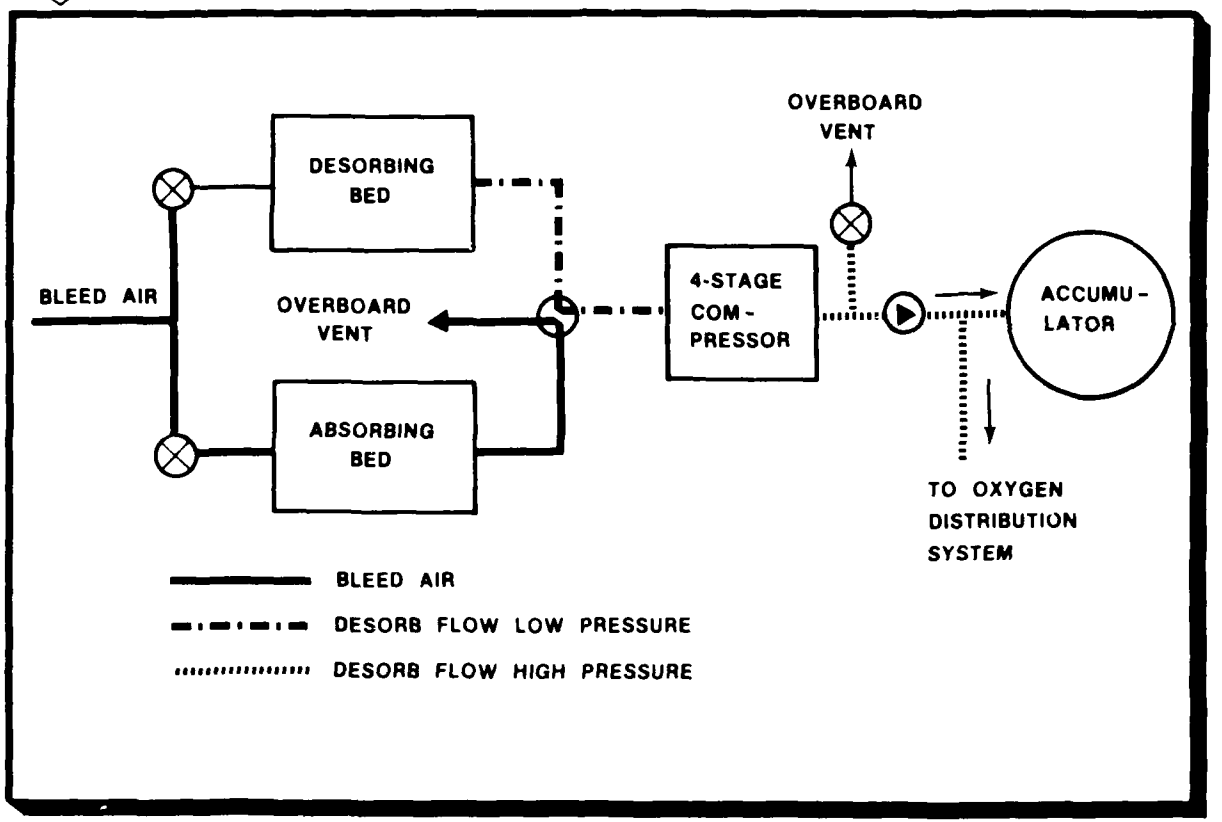

FIGURE 3 - FLUOMINE OXYGEN SYSTEM

SIMPLE CONCENTRATOR DESIGN

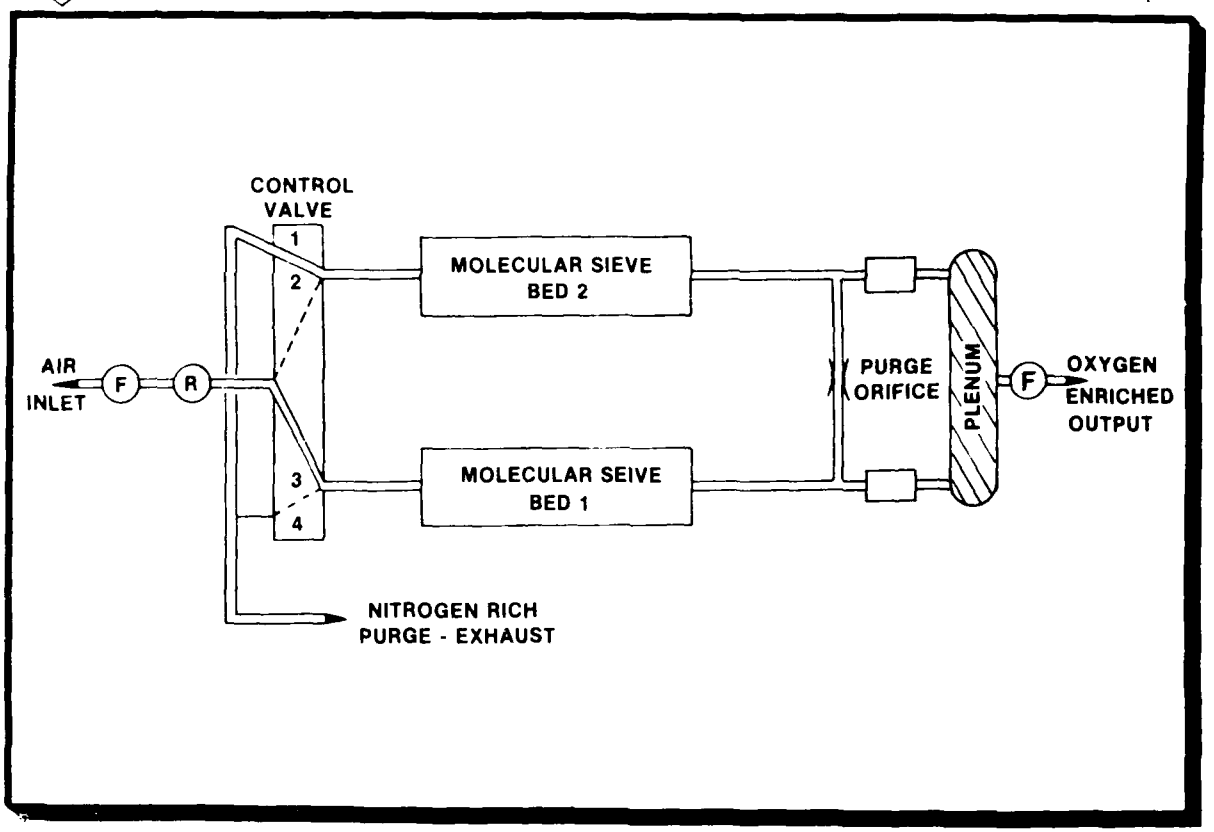

FIGURE 4 - MOLECULAR SIEVE OXYGEN
GENERATOR DESIGN

LIQUID OXYGEN VS MOLE SIEVE OBOGS

	VOLUME	WEIGHT
MS OBOGS	0.88 cu ft	35 lbs
5 LITER CONVERTER	1.06	25
10 LITER CONVERTER	1.6	36

FIGURE 5 - VOLUME AND WEIGHT OF OLOGS

FIGURE 6 – THE MOLECULAR SIEVE OXYGEN GENERATOR PROTOTYPE

SUBSTANTIAL COST SAVINGS

(300 UNITS, 10 YEAR PERIOD)

	LOX	OBOGS
AIRCRAFT EQUIPMENT	$450,000	1,860,000
* SUPPORT EQUIPMENT	75,000	6,000
BASE SUPPORT		
LOX (PURCHASED)	864,000	
LABOR	8,868,000	376,000
DEPOT REPAIR		
UNITS	1,500,000	1,460,000
* SUPPORT EQUIP	75,000	
	11,832,000	3,702,000

SAVINGS: 8,130,000 TOTAL

* DOES NOT INCLUDE LOX GENERATORS SAVINGS

FIGURE 7 - COST COMPARISONS OF LOX VERSUS OBOGS

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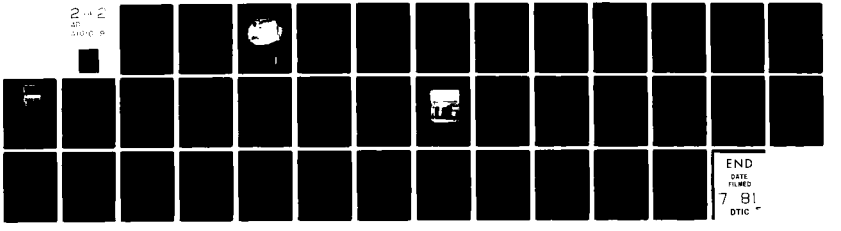

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MS OBOGS PRODUCT GAS FLOW RATES

SUPPLY AIR PRESSURE	AIRCRAFT ALTITUDE	CABIN ALTITUDE	PRODUCT GAS FLOW RATE
8 PSIG	G.L.	G.L.	13.1 1PM
15	10,000 ft	7,500	26.2
25	20,000	8,000	39.3
		12,000	39.3
		20,000	39.3
40	30,000	12,000	52.4
		16,000	52.4
		30,000	52.4
60	40,000	16,500	78.6
		22,000	78.6
		40,000	78.6
60	44,000	18,000	78.6
		44,000	78.6

TABLE I - PRODUCT GAS FLOW RATES

MOLECULAR SIEVE OXYGEN GENERATION SYSTEM: CONTAMINANT STUDIES

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Brooks Air Force Base TX 78235

The molecular sieve method of generating oxygen enriched air for onboard aircraft is currently being actively pursued, not only by the United States but other countries as well. Thus far laboratories have been the prime place for evaluation of the molecular sieve system and, during this evaluation, have been provided with essentially clean air. In aircraft, however, the molecular sieve system will be supplied with engine bleed air which may not always be totally free of contaminants. Recent laboratory studies conducted at the USAF School of Aerospace Medicine suggest that the molecular sieve system is an effective filter for many contaminants, including water, as noted by the lack of impairment of performance.

INTRODUCTION

The concept of inflight generation of breathing gas is attractive for military aircraft from the standpoint of logistics; safety, and cost. The molecular sieve method of generating an oxygen enriched air breathing gas for application in training and tactical aircraft is being actively pursued not only by the armed forces of the United States but by other countries as well. Molecular sieve oxygen generating systems have received extensive laboratory evaluation (1, 2, 3, and 4) and currently are being flight-tested both by the U.S. Navy and U.S. Army. Evaluation and physiological assessment of the molecular sieve system in the laboratory have been generally conducted with clean compressed air. In aircraft, however, the molecular sieve generator is supplied with engine bleed air which may not always be totally free of contaminants. Laboratory studies conducted at the USAF School of Aerospace Medicine have demonstrated that low molecular weight compounds pass through a molecular sieve bed into the breathing gas. The concentrations of the contaminants appearing in the output breathing gas are, however, much lower than that in the supply air and have been shown to be directly related to the output demand flow of the oxygen generator.

METHODS AND MATERIALS

The Molecular Sieve System--The molecular sieve oxygen generator system (Fig. 1) used in this investigation was manufactured by the Bendix Corporation, Instrument and Life Support Division, Davenport, Iowa. This system is similar to the system being tested by the U.S. Navy in the AV 8-A "Harrier." A schematic of the Bendix "Harrier Unit" is shown in Fig. 2.

The system utilizes a pressure swing adsorption process (PSA) with two beds and a gaseous back purge for bed regeneration. Bleed air is alternatively admitted to the molecular sieve beds through a filter, pressure regulator, and rotating inlet valve (6 rpm). As the pressure front propagates through the bed, the air is separated into several constituents, including oxygen/argon. A portion of the oxygen/argon-rich product passes through a check valve and into a 1 liter plenum prior to delivery to a demand regulator, while the remainder of the oxygen/argon-rich product and nitrogen are utilized to purge the molecular sieve beds.

Molecular Sieve Sorbent--Chemically, molecular sieves are crystalline aluminosilicate compounds called zeolites with the general formula MN_2O-Al_2O-$nSiO_2$-mH_2O, where M is calcium strontium, or barium, and N is sodium or potassium (5). The ability of the molecular sieve to separate gas mixtures into components is based primarily on the porous structure of the zeolite crystal. The highly uniform pores, with dimensions in the molecular range, are formed by heating the fresh zeolite which loses its water of hydration and undergoes lattice shrinkage. Pore size can be changed by varying the concentration of the alkali metals in the crystal. Gas mixtures can be separated into identifiable constituents on molecular sieve on the basis of physical dimensions (molecular diameters). Polar molecules are retained on the molecular sieve by Van Der Waal's forces rather than chemical bonding. Thus, the process can be reversed by fairly mild changes in temperature or pressure.

Experimental Setup--The experimental setup shown schematically in Fig. 3 was assembled to evaluate the molecular sieve performance when challenged by contaminants. The unique characteristic of this system is its ability to generate contaminants from a liquid source for hours at a time at pressures greater than ambient. This capability is made possible through the use of Vycor® 7930 porous glass tubes. The Vycor® glass tube acts as an evaporation tube whose pores have a diameter in the order of 40 angstroms. The pore size of 40 angstroms makes it possible to have the liquid contaminant on one side of the glass tube at ambient pressure while on the other side pressures up to 175 atmospheres can be applied without forcing the liquid contaminant out of the pores. Thus, such a system provides an excellent means of contaminating air at high flows for an unlimited time. In addition, the steel plenum which has a volume of approximately 300 liters can be heated to a temperature of 125°C. Gaseous contaminants; e.g., methane, ethane, were fed directly to the steel plenum from a pressurized source through a

regulator and rotameter in order to maintain a constant flow. It was possible to, therefore, generate a contaminant in air in the ppm range (10-1000 ppm) at flows of 300 liters/min and at temperatures up to 125°C. This contaminated air was then fed directly into a molecular sieve oxygen generating system for system evaluation.

Figure 1
The Bendix Harrier Molecular Sieve Oxygen Generating System

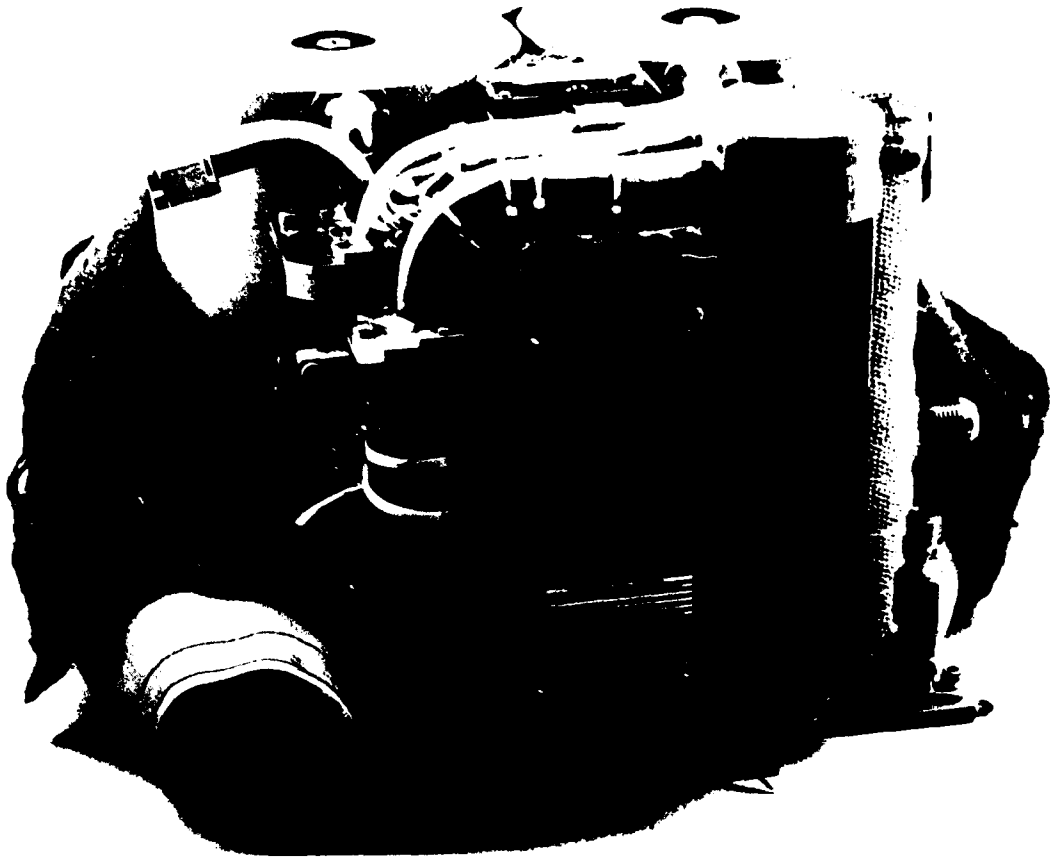


Figure 2
Schematic of the Molecular Sieve Oxygen Generating System

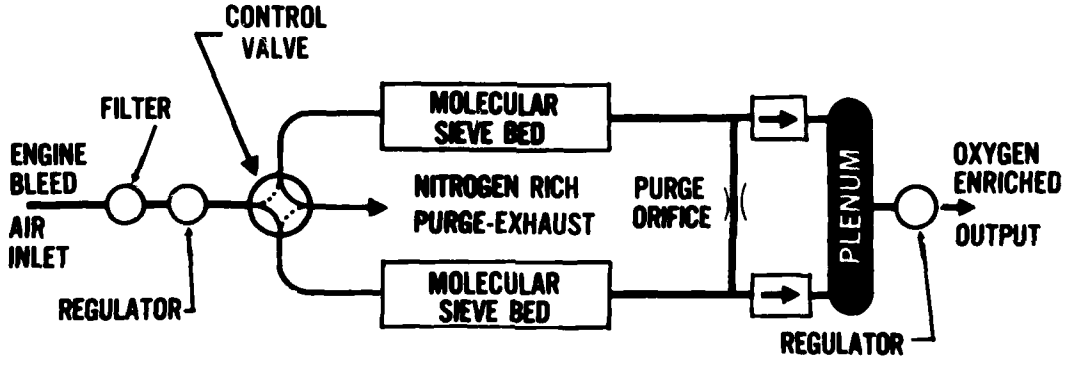
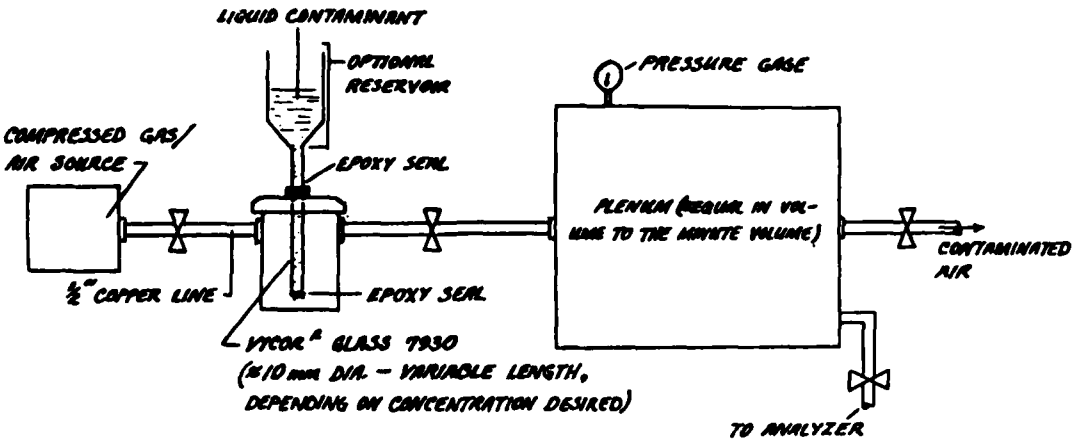


Figure 3. High Volume Contaminant Generating System



The analysis of the contaminant level in air being fed to the molecular sieve unit and the contaminant level in the product gas and exhaust gas were measured with a Beckman Model 402 total hydrocarbon flame ionization analyzer.

Samples of gases taken for analysis included the inlet gas to the molecular sieve unit, the molecular sieve purge gas, and the molecular sieve product gas. In order to determine the total amount of contaminant at the inlet exhaust and product of the molecular sieve system, accurate flow measurements were made. Flow was measured by either dry gas meters or rotameters.

The ingestion of water into the molecular sieve was included in this study, not only from the point of view as a breakdown product of aircraft fuels/lubricants, but also from the intake of water when flying through a rainstorm, or engine washing as is the case with AV-8A. Water was introduced into the molecular sieve inlet air by the use of a pneumatically operated 50 cc syringe.

EXPERIMENTAL PROTOCOL

The objective of this test program was to determine composition of the product gas delivered by the molecular sieve system as a function of contaminants, contaminant concentration, inlet temperature, and flow at a constant inlet pressure. Composition (O_2 and contaminant) of the product gas was measured at each flow as was that of the purge gas and the inlet gas.

Contaminants selected thus far for this program include methane, ethane, hexane, methanol, methylethylketone, and water. These contaminants were selected primarily on the basis of a previous USAF report by K. L. Paciorek, et al. (6). This effort characterized breakdown (450°C) products of turbine lubricating oils, hydraulic fluids, and heat-transfer fluids (Table 1).

TABLE 1. SOME VOLATILE PRODUCTS FORMED BY AIRCRAFT FLUIDS ON EXPOSURE TO STEEL SURFACE AT 450°C

	LUBRICATING OILS		HYDRAULIC FLUID
	TURBO OIL 2389 (mg/g)	BRAYCO 880X (mg/g)	BRAYCO 756E (mg/g)
CO	8.6	8.5	4.4
H_2O	49.3	67.6	40.4
CO_2	26.8	25.5	12.8
CH_4	0.10	0.09	0.02
C_2-SPECIES	6.15	6.82	1.14
C_6-SPECIES	5.53	3.35	0.789
C_8-SPECIES	3.13	8.37	1.43
PROPIONALDEHYDE	2.11	2.27	0.058
METHYLETHYLKETONE	2.29	1.51	1.19
METHANOL	1.19	3.76	0.942

The effect of high ambient temperature on the molecular sieve unit was also undertaken in this study, in order to more clearly define the optimum temperature range of the molecular sieve beds to maintain effective air separation.

a variety of contaminants. In addition, the contribution of contaminants from the engine itself; i.e., turbine engine oil, may result in decreased efficiency of the system. Thus far, only a very few contaminants have been studied.

TABLE V. CHALLENGE - METHYLETHYLKETONE (MEK)
Inlet Pressure 28 psig Temperature OBOGS 28°C
FLOWS MEASURED AT ATP

INLET CONTAMINANT MEK ppm (mg/min)		PRODUCT GAS FLOW lpm	% O_2	MEK ppm	EXHAUST GAS FLOW lpm	MEK (mg/min)	
0		10	92.1	0	268	0	
81	(65.5)	10	92.3	0	269	73	(56.9)
79	(64.1)	30	56.8	0	260	77	(58.0)
80	(75.4)	70	37.8	0	255	82	(60.6)

TABLE VI. CHALLENGE - HEXANE
Inlet Pressure 28 psig Temperature OBOGS 22°C
FLOWS MEASURED AT ATP

INLET CONTAMINANT HEXANE ppm (mg/min)		PRODUCT GAS FLOW lpm	% O_2	HEXANE ppm	(mg/min)	EXHAUST GAS FLOW lpm	HEXANE ppm	(mg/min)
0		10	91.5	0		268	0	
61	(58.7)	10	91.7	<1		268	44	(45.3)
60	(61.6)	30	54	4.5	(0.5)	262	47	(43.3)
56	(60.4)	70	38.2	13	(3.2)	252	48	(43.6)
188	(176)	10	91.4	2	(0.07)	257	170	(151)
190	(191)	30	52	22	(2.3)	259	165	(149)
186	(210)	70	37.6	41	(10.0)	257	178	(160)

The result of the ingestion of water into the system (Table VII) indicates that the product gas oxygen concentration and dew point were not changed when up to 600 ml water were introduced over a 30 min period. Most importantly, the coalescent filter trapped and eliminated most of the water (62%), through the fixed orifice, before the water could enter the molecular sieve beds. That water which entered the beds was eliminated from the beds by the purge, depressurization cycle. This was particularly evident by the visible water vapor/droplets in the exhaust gas. The ingestion of the 600 ml of water by the system did not alter bed efficiency or performance indicating that the water did not migrate down the beds any appreciable distance.

TABLE VII. CHALLENGE - WATER
Inlet Pressure 28 psig Temperature OBOGS 30°C
FLOWS MEASURED AT ATP

INLET CONTAMINANT WATER ml (ml/min)		PRODUCT GAS FLOW lpm	% O_2	DEW POINT (°C)	COALESCENT FILTER WATER COLLECTED
0		10	92.3	-28	0
0		70	35.6	-28	0
300	(10)	10	92.5*	-27	178 ml
300	(10)	70	35.6*	-28	190 ml
600	(20)	10	92.6*	-27	389 ml

* After water challenge

This study concerning the effects of some contaminants on the Bendix molecular sieve oxygen generating system can be summarized by the following:

(1) Filtering effectiveness is a complex matter making it difficult to generalize.

(2) Further work is required to define the filtering effects of molecular sieve; e.g., water, oil aerosols, and organo phosphates.

(3) This limited study suggests that the molecular sieve is an effective filter for many contaminants, including water as noted by the lack of impairment of performance (oxygen separation).

(4) Temperature of the inlet air to the molecular sieve unit should lie between 0°C and 70°C.

Additional studies are slated to determine what level of water ingestion of the molecular sieve beds is necessary to affect the product gas/bed performance.

The effect of increased temperature of the input air on the molecular sieve product gas is depicted in Figs. 4, 5, and 6 as a function of minute volume. At 10 lpm volume, the oxygen concentration drops only slightly when the temperature is increased (0.5°C/min) from ambient to approximately 70°C. As the temperature of the inlet air is increased from 70°C to 120°C, a dramatic decrease in the oxygen concentration is observed. As the heat is removed, a hysteresis effect is noted which almost certainly is due to the large heat sink represented by the molecular sieve and associated materials. This same effect also holds true when the minute volume is increased to 30 lpm (Fig. 5). However, at a minute volume of 70 lpm (Fig. 6), the change in the oxygen concentration is not as great, primarily due to the lower initial concentration of oxygen in the product gas.

Figure 4
CHALLENGE - HEAT

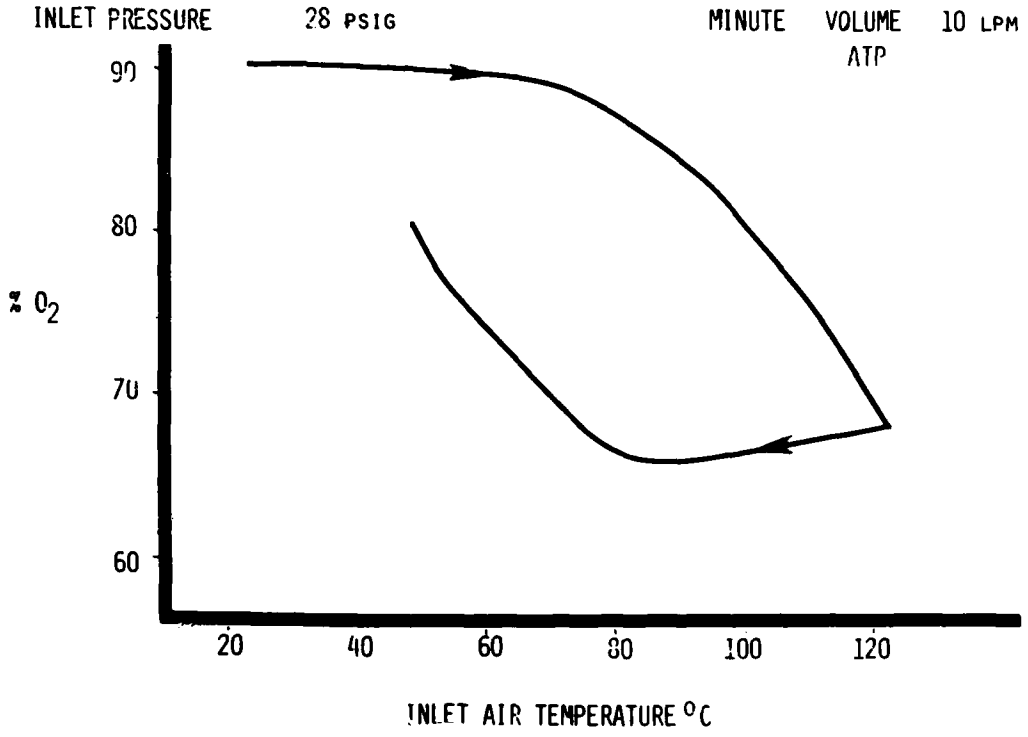
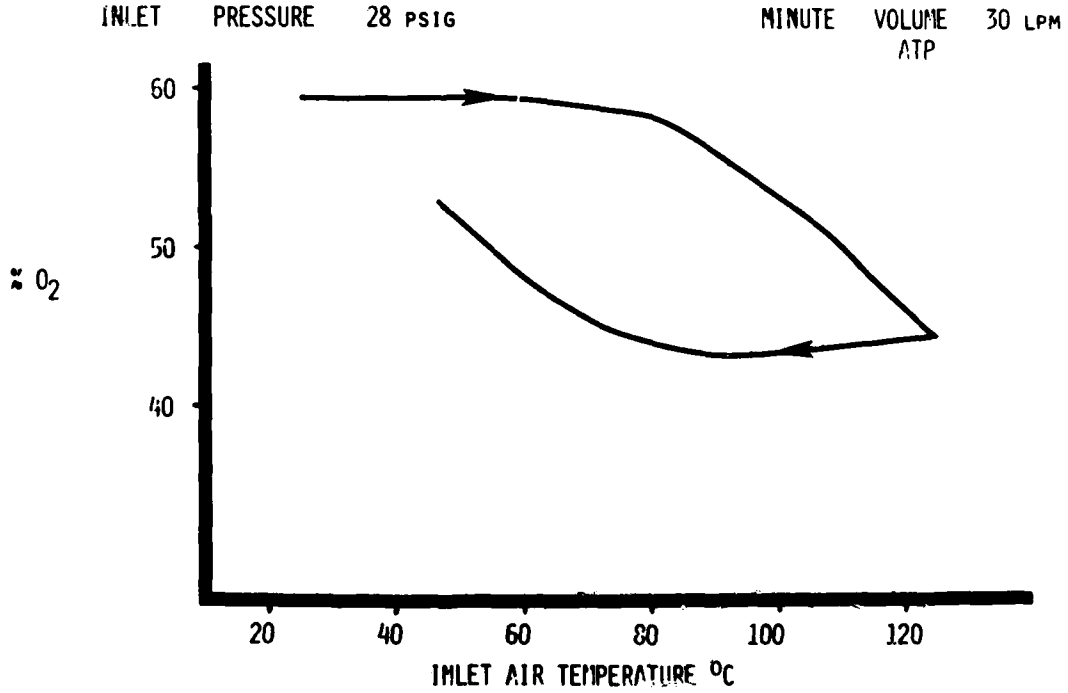


Figure 5
CHALLENGE - HEAT



RESULTS AND DISCUSSION

Challenging the molecular sieve unit with low molecular weight contaminants; i.e., methane and ethane has resulted in these compounds breaking through the beds and appearing in the product gas (Tables II and III). The concentrations of the contaminants appearing in the product gas are, however, much lower than that in the inlet supply, and the concentrations are related to the minute volume.

TABLE II. CHALLENGE - METHANE
Inlet Pressure 28 psig Temperature OBOGS 28°C
FLOWS MEASURED AT ATP

INLET CONTAMINANT		PRODUCT GAS				EXHAUST GAS		
METHANE ppm	(mg/min)	FLOW lpm	% O_2	METHANE ppm	(mg/min)	FLOW lpm	METHANE ppm	(mg/min)
0		10	81.6	0		248	0	
131	(21.9)	10	81.4	0		246	134	(21.6)
141	(24.5)	30	48.5	1	(0.02)	236	167	(25.7)
150	(26.9)	70	33.1	45	(1.3)	205	184	(24.7)

TABLE III. CHALLENGE - ETHANE
Inlet Pressure 28 psig Temperature OBOGS 23°C
FLOWS MEASURED AT ATP

INLET CONTAMINANT		PRODUCT GAS				EXHAUST GAS		
ETHANE ppm	(mg/min)	FLOW lpm	% O_2	ETHANE ppm	(mg/min)	FLOW lpm	ETHANE ppm	(mg/min)
0		10	91.8	0		270	0	
85	(19.3)	10	91.4	0		269	92	(20.1)
85	(20.1)	30	52.3	0		260	103	(21.9)
85	(22.0)	70	35.9	20	(1.08)	250	105	(22.2)

It is interesting to note, when measuring (ppmv) concentration of the low molecular weight compounds in the product gas and exhaust gas and comparing this value to the inlet concentration, that the molecular sieve unit appears to be generating contaminants. This erroneous conclusion can be quickly allayed by conducting a mass balance considering the rate of introduction of contaminant into the air supply and the amount of contaminant in the product and exhaust gases per unit time. A mass balance is noted when product gas and exhaust gas contaminants are summed and compared to the amount of contaminant introduced to the system. In addition, this mass balance indicates that the low molecular weight compounds are weakly adsorbed on the molecular sieve allowing a small portion of the contaminant to break through the bed to the product gas while the remainder of the contaminant is purged from the bed and appears in the exhaust gas. As the flow of product gas is increased, contaminant concentrations are also increased. This is primarily due to the decrease in the amount of gas available for purging the beds.

Polar molecules such as methanol and methylethylketone do not break through the molecular sieve beds at these concentrations (Tables IV and V). The data indicates that the adsorptive forces (Van der Waal's) of these compounds are much stronger than those of the low molecular weight compounds, but still weak enough so that the process can be reversed by the reduction of the partial pressure of the contaminants which occurs when the bed is vented to exhaust and purged. Mass balance shows that a small portion of the contaminant is retained by the molecular sieve. The contaminant retained on the bed rapidly fell to zero when the contaminant was removed from the inlet air.

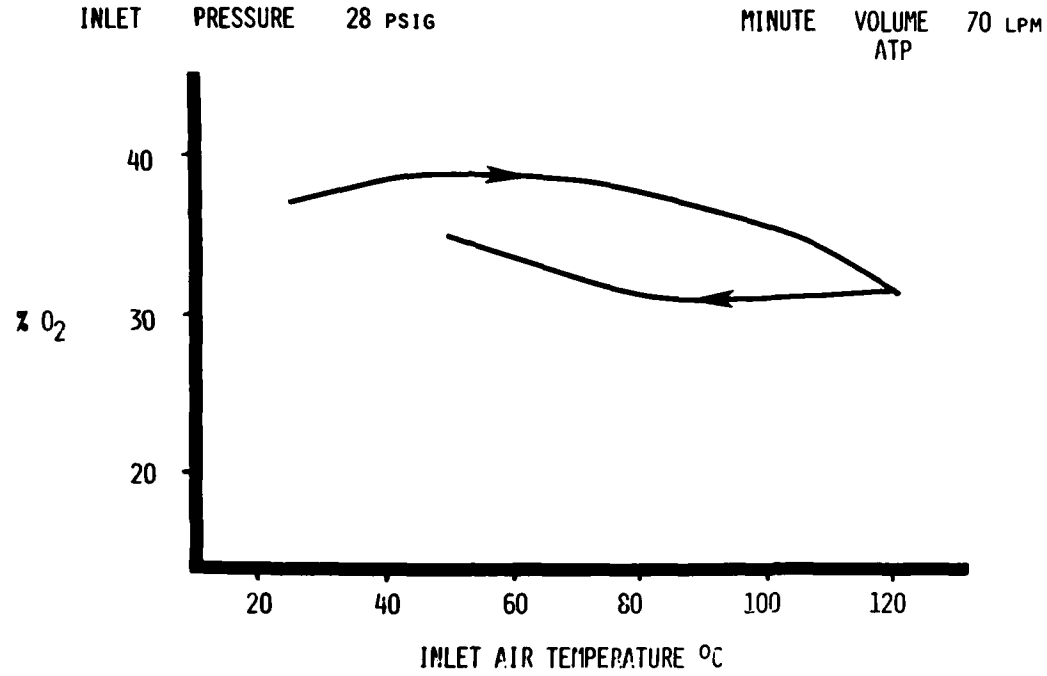
TABLE IV. CHALLENGE - METHANE
Inlet Pressure 28 psig Temperature OBOGS 22°C
FLOWS MEASURED AT ATP

INLET CONTAMINANT		PRODUCT GAS			EXHAUST GAS		
METHANOL ppm	(mg/min)	FLOW lpm	% O_2	METHANOL ppm	FLOW lpm	METHANOL ppm	(mg/min)
0		10	91.6	0	271	0	
138	(50.1)	10	91.6	0	267	135	(47)
137.7	(51.2)	30	57.5	0	254	146	(46.4)
133	(54.3)	70	36.2	0	242	154	(48.5)

There are also compounds that are neither weakly nor strongly adsorbed on the molecular sieve. Hexane (e.g., as illustrated in Table VI) breaks through the molecular sieve beds much like the low molecular weight compounds; however, there is no mass balance which is similar to the adsorption of more polar compounds.

Additional organic contaminant studies are required to fully define and understand the molecular sieve system. Since this system utilizes engine bleed air, the source of the air and its quality become important, particularly when the source of air contains

Figure 6
CHALLENGE - HEAT



CONCLUSION

Results of this evaluation indicate that the molecular sieve system can adequately filter contaminants or in the case of low molecular weight compounds; i.e., methane and ethane, reduce the contaminant concentration in the product gas to what can be considered an acceptable level. The higher molecular weight compounds and polar compounds are not expected to break through the molecular sieve beds barring some gross contamination over an extended period of time, although certain compounds may break through; i.e., hexane.

The temperature of the inlet air is a critical factor influencing the performance of a molecular sieve. As noted by Miller, et al. (1), the oxygen concentration in the product gas was greatly decreased as the inlet temperature and the molecular sieve unit were lowered from about -20°C to -54°C.

Present studies, which considered higher air inlet temperatures to the molecular sieve system, also noted a decrease in the product gas oxygen concentration as temperatures were increased above 70°C. Therefore, for the system to maintain effective air separation, the molecular sieve beds and inlet bleed air must be maintained in the temperature range of 0°C to 70°C.

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AIRSCAN: AN ULTRASENSITIVE TRACE AIR IMPURITY ANALYZER FOR USE IN TOXIC AVIATION ENVIRONMENTS

by

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ABSTRACT

An advanced air analyser is described that is capable of detecting a wide range of pollutants in ambient air at concentrations of well below 1 part-per-billion. The system employs a combination of photoionization detection with gas chromatography using air as the carrier gas; it is fully field portable and accepts a directly introduced sample of air without any need for time-consuming pre-concentration procedures.

Applications for the instrument, the commercial embodiment of which is now being marketed under the trade name Airscan, include the monitoring of all manner of environments including those within aircraft and spacecraft as well as external environments which may be contaminated during fuelling operations or by accidental emissions from specialized ordinance. Hydrazine is of particular current relevance.

Further applications include the monitoring of human exhaled breath in cases where there has been an unquantified exposure to toxic compounds. Up to now such exposures have been traditionally difficult to monitor at low levels; however the extreme sensitivity of the Airscan system has made it possible to detect breath metabolites in extremely small concentrations.

Also described is a unique field portable data analysis system which has been developed to the prototype stage as a companion unit for Airscan. The objective for this instrument is to provide each Airscan user with a library of compounds of special interest. The output from the Airscan chromatograph is fed directly into the analyser which records the chromatographic peaks in terms of their retention time and area and then proceeds to scan its library for correspondence. When a compound is identified, the analyser prints out the name on a 20-character wide tape together with its corresponding concentration in the air. Up to 75 different compounds can be analysed in any given run in the present configuration.

INTRODUCTION

A requirement exists in aviation technology for an instrument that can be rapidly used to assess the degree of hazard to aircrew and ground support personnel from exposure to low concentrations of airborne toxic chemicals that either emanate from liquids and solids necessary to aircraft and their maintenance or are produced as a consequence of aircraft operation.

A recent review (1) has documented the hazards of toxic gases and vapours in the aviation environment to both aircrew and ground support personnel. The main sources of toxic gases and vapours in flight are products of combustion, either from the exhaust of the engine or overheating and fire: aviation fuels, lubricants and hydraulic fluids; anti-icing, anti-detonant and coolant fluids: fire extinguishing agents: refrigerents; ozone and insecticides, herbicides and agricultural chemicals. All of these are hazards in the ground support environment as well and with the increasing use of toxic substances such as hydrazene as fuel for high performance aircraft, greater concern must be expressed for monitoring of trace concentrations of these chemicals on the ground and in the air. The totally enclosed environments of spacecraft also present new challenges in the maintenance of environmental quality.

Numerous techniques have been developed for the detection of gaseous and vapourous contaminants in air. Instrumentation that can be used in the field may involve such different detection techniques as infra-red absorption, automated colormetric analysis, flame ionization detection (FID) or photoionization detection (PID) used in conjunction with gas chromatography. In the majority of cases, these and other techniques can be used only with difficulty in attaining stable performance below the 0.1 part per million (ppm) contamination level.

PID has shown promise as a potentially sensitive technique but, until recently, it has suffered from a number of problems in the attainment of its theoretical sensitivity due to interference by both oxygen and water vapour in the air and its general stability. The instrument to be described in this paper employs an advanced PID system which has successfully overcome many of these problems.

In principle, PID involves irradiation of sample with short wave length ultraviolet (UV) light (in the region between 2000 and 1000 Angstroms). Such radiation has a photon energy which can selectively ionize many compounds of environmental interest but cannot generally ionize the matrix gas in which the pollutant is dispersed (this matrix is generally air but may also be an inert gas). When such a detector is attached to the end of a gas chromatograph column, the essence of a sensitive air impurity analyser is found.

THE AIRSCAN SYSTEM

A PID detector has been developed which differs from those currently available in several important respects; these include a higher intensity UV light source with a more distributed emergent flux, a radically redesigned ion chamber and improved electronics. The detector is being incorporated into a field portable gas chromatograph and is being sold under the trade name Airscan.

The instrument, which is shown in Figure 1, is self-contained with re-chargable battery power sufficient for a day's operation and an internal carrier gas supply which can last for up to ten days. The dimensions are approximately 41 cm (16") X 25 cm (10") X 22 cm (9") and the weight is 11 kg (24 lbs).

Because of the novelty of the detector, it is possible for the instrument to operate with excellent sensitivity using air as the carrier gas. This has not been previously possible with other systems because an effect known as "quenching" occurs in the presence of oxygen which drastically reduces sensitivity. The abiiity to operate with an air carrier allows large volume air samples (up to 1 ml) to be injected without the occurrence of the usual injection artefact when an inert gas carrier is employed.

Under the above conditions, Airscan can offer sensitivities to airborne pollutants down to 0.1 parts per billion (ppb). Some of the compounds which can be detected to this level are benzene, toluene, xylene, vinyl chloride, chloroform isoprene, propane, ethylene, hydrogen sulphide, carbon disulphide, mercaptans, methyl chloride, halothane, nitric oxide, ethyl acrylate, benzene chloride and hydrazene. Many of these compounds are important in the aviation environment context.

In situations where such extreme sensitivity is not required Airscan offers excellent stability and rapid cold start capability, often within five minutes.

MATTERS RELATING TO SENSITIVITY

The primary function of Airscan is to analyse air samples directly for ionizable compounds. As has been intimated, this can be done with unprecedented sensitivity when required. Control over sensitivity allows direct sampling to yield a dynamic operating range of 10^6 or, in terms of concentration, between 0.1 ppb and 100 ppm.

When samples are to be taken in the ppm range, the instrument is set to a high attenuation factor (X100) and a small-volume air sample is injected. The electronic attenuation can be adjusted within the range 1-100, while the sample volume can readily be varied between 1 microlitre and 1 millilitre. In this way we can define an Instrument Sensitivity Factor, S, such that:

$$S = \frac{\text{Sample volume in microlitres}}{\text{Attenuation factor}}$$

This factor is a relative one and particular to Airscan, for which it is useful in comparison to data. Thus S can vary numerically between 1000/1 = 1000 and 1/100 = 0.01. Obviously, further gain and/or attenuation can be applied (usually by using the chart-recorder amplifier) and S factors of 5000 have frequently been employed.

Work in the occupational health area often involves monitoring of levels close to the ppm range and the question may be asked "Why use sub-ppb sensitivity when working in the ppm range?" The answer to this comes from examination of the existing technology. Most of the available field-portable instruments now in use have difficulty in performing at the ppm level; considerable instrument noise is evident and long time periods are required to ensure stability. The sensitivity of Airscan ensures that, when ppm levels must be measured, absolutely no instrument noise is evident and stable operation is reached within five minutes of a 'cold-start'. When Airscan is used measure sub-ppb levels of contamination, some noise is evident and longer stabilization times are required.

To perform analyses with the Airscan system, very small sample volumes are required, 50 ul being a typical value, in contrast to the multi-litre volumes of air usually required for laboratory concentration either cryogenically or by passing through a charcoal collection tube containing an appropriate adsorption medium for later extraction and conventional analysis. The advantage of this small-volume sampling feature can be realized in a new approach to time-weighted average (TWA) personnel monitoring. A tiny metal vessel can be evacuated and fitted with a 'critical orifice assembly' (3) which permits the controlled entry of work-place air into the vessel so that the internal pressure will reach 1/2 atmosphere in a period of eight hours. The contents reflect the true exposure that a worker may have had to airborn chemicals. With Airscan, the sample may be quickly analyzed in the field or laboratory and the internal volume of the collection vessel need be only 1 ml or less. Conventional time-weighted averaging can still be accomplished using Airscan; in this case, a small collection bag may be slowly filled over a period of a shift and a TWA sample withdrawn through a syringe port at any time without impact on the ongoing collection.

GAS CHROMATOGRAPH COLUMNS

Airscan employs an ambient temperature column which is mounted internally; a wide range of suitable columns may easily be installed. Two major limitations of this feature exist. One is that as the ambient temperature changes, there will be a corresponding change in column parameters; most notably, retention time will change and, where this occurs, calibration will be necessary. While this effect does introduce some problems, it has been found that these are easily overcome and some efficient methods of preparing and storing standards have been developed for this purpose. Further

simplification of this problem has come with the introduction of a computer-managed signal analysis calibration system which is able to refer to a built-in library of compounds for identification by name and quantification of their level of presence, while taking temperature-drift into account.

The second limitation of ambient columns is that heavy, high boiling point compounds have very long retention times and the peaks which ultimately emerge from the column will be greatly reduced in magnitude due to column losses. While there are some compounds which are intractable to the ambient technique, a number of means are available for overcoming the problem in many cases. Where a high boiling point compound is specifically being sought, a short, small diameter column may be employed most effectively. A 25 cm (10") 1.5 mm (1/16") diameter packed SE-30 column has been used with great effect to detect terpene compounds, the boiling points of which are close to 200°C. Ethylene glycol dinitrate, another high boiling point compound and a component of dynamite, is also readily detected.

Further work with high boiling point compounds at ambient temperatures is being carried out using the new generation of fused quartz capillary columns. With these, no splitting is required as Airscan can operate readily at flow rates of 5-10 ml/minute. High boiling point compounds elute rapidly with these columns with excellent resolution, even at ambient temperatures.

For specific situations, such as the direct analysis of human breath in which high levels of water vapour are injected with each sample, it is important to choose a non-degradable liquid phase. Satisfactory results have been obtained using SE-30 for non-polar compounds and Carbowax 20M for moderately polar compounds.

RESULTS

(a) Dynamic range:

Figure 2 shows the result of a direct injection of a simulated workplace environmental sample, containing 1 ppm of benzene, some trace light hydrocarbons and a small amount of toluene. In this chromatogram the benzene peak is by far the largest and the majority of the remaining compounds represent concentration levels in the range of 0.1 to 0.2 ppm. At an S setting of 10, no noise was evident. The sample volume injected was 100 ul.

Figure 3 shows the same sample run at an "S" value of 200. The benzene peak is now truncated and many of the other peaks are off-scale as well; however, new peaks appeared the sizes of which correspond to concentrations in the low ppb range. There was no evidence of noise on the trace.

Figure 4 shows an environmental sample (suburban air) containing 2 ppb of benzene. The S factor was set at 5000 and, at this sensitivity, a certain amount of instrument noise was apparent. The presence of benzene in suburban air is not surprising in view of the high levels of this compound which are present in the new 'Super' grades of unleaded gasoline: evaporation from car gas tanks is the most likely source.

(b) Compounds of Environmental Concern:

Figures 5 through 7 illustrate the analysis of several compounds with the corresponding sampling parameters given. These include pinene, limonene, arsine, phosphine, methylene chloride, n-hexane and benzene.

(c) Analysis of Human Breath:

Airscan is capable of detecting a range of trace compounds present in human breath which may be of metabolic or environmental origin. There compounds are present in very small concentration in relation to the gross concentrations of carbon dioxide and water.

A single experiment was performed to examine the breath-levels of ethanol in a subject who had taken a dose of 0.3 gm/kg body mass. Utilizing Airscan analysis it was possible to track the alcohol in the subject's breath for a period of 3 1/2 hours after ingestion. The ultimate limitation was the subject's own endogenous alcohol level which was approached after this length of time. The sampling technique involved the subject taking several deep breaths, holding his breath for 30 seconds, and then taking a syringe sample of 50 ul directly from his mouth as he exhaled the last portion of breath. This sample was then injected directly into an Airscan instrument, fitted with a 50 cm Carbowax 20M column. Figure 8 shows the data obtained, arranged sequentially through time. Figure 9 shows a plot of ethanol level in the breath (in ppm) as a function of time. Such a sampling procedure may be simplified with the aid of a sample loop connected to the injection port of the Airscan.

Calibrations were carried out at four points: 18, 36, 52 and 72 ppm ethanol in air. The analytical precision was tested for this compound (Figure 10) and found to be within ±1.5%. A trace from the background air breathed is included in Figure 11 and, for comparison, a trace from a subject who had taken no alcohol is also shown in Figure 11.

(d) Real-Time Monitoring:

The Airscan can be operated in a mode in which the carrier gas supply is replaced with a vacuum pump and the column removed. In this mode of operation, the sample is drawn from the environment through the ionization chamber and the instrument then measures the level of the Total Ionizables Present (TIP). In this mode, Airscan compares very favourably with some of the other total organic detectors which are now on the market which cannot usually detect less than 1 ppm of contamination. Figure 12 shows Airscan results for the TIP mode; alternating analyses between clean bottled air (Medical Grade) and laboratory air at approximately 1 ppm TIP. Note that there is no noise on the trace when clean air is being monitored but that fluctuations are apparent in the laboratory air,

presumably due to eddies in the concentration of airborn impurities. The detection threshold in the TIP mode is better than 20 ppb.

AIRSCAN DATA SYSTEM

A prototype portable data system has been developed for use with Airscan. This system is designed to measure peak retention time and peak area and to carry out the management of the calibration function, thereby simplifying the use of ambient columns. On computer prompting, a single component gas standard is injected into the Airscan by the operator. The computer notes the parameters of this standard run and proceeds to normalize all the data (based on retention time, response factor and drift-coefficient for the particular compound) contained within a semiconductor library comprised of up to 72 relevant compounds. Thus, the single point calibration serves to correct all data for drift. Upon completion of this simple procedure, the sample under study may be introduced. The unit then provides a record of the run in which the identified compounds are listed and their levels of presence (in ppm) are recorded on a 20-character wide thermal-printed tape. If a peak has appeared which does not correspond to anything held in the library, the computer will simply print "UNKNOWN" and give the retention time and the peak area. At this point a second memory may be plugged in and the attempt at identification repeated. Figure 13 shows a typical record from the prototype instrument. Figure 14 shows the present instrument in operation.

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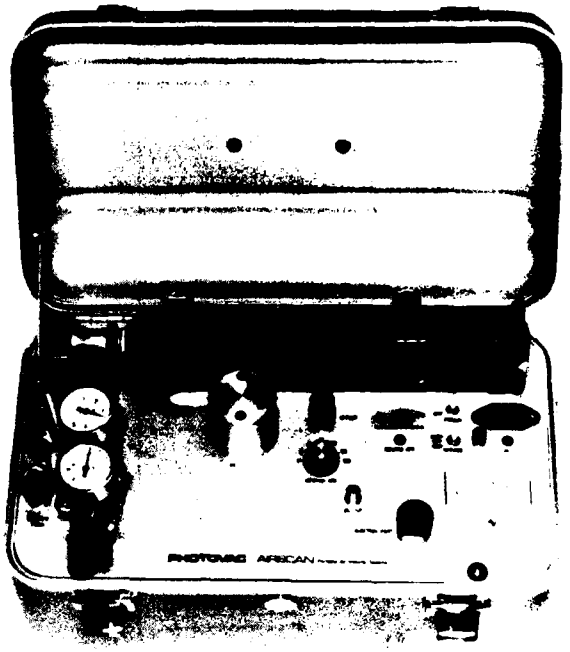

Fig.1 The Airscan system

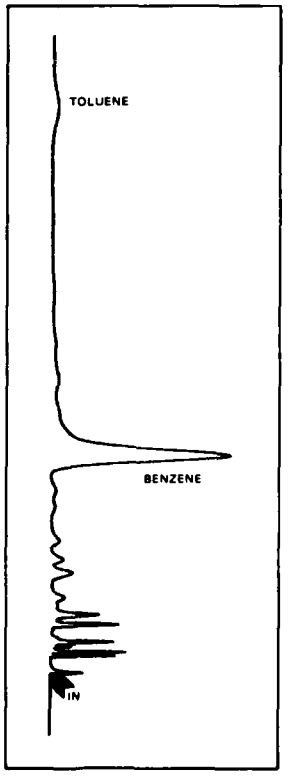

Fig.2 Results of Airscan measurement on sample of simulated workplace air contaminated with 1 part-per-million of benzene vapour as the major component, along with some minor hydrocarbon components. Instrumental sensitivity factor is 10

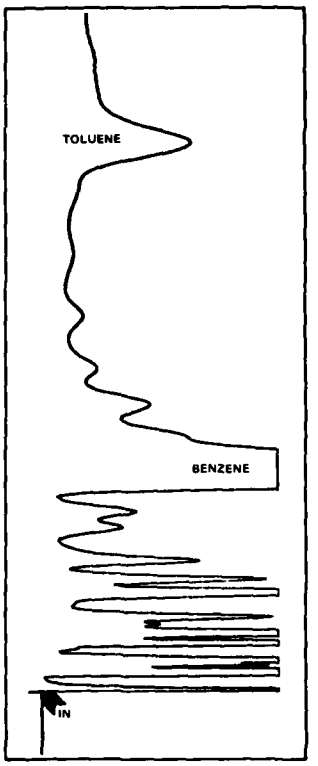

Fig.3 Results of Airscan measurement on the same mixture shown in Figure 1 but with the instrumental sensitivity now set at 200. Note the total absence of baseline noise and the profusion of peaks in the low part-per-billion range

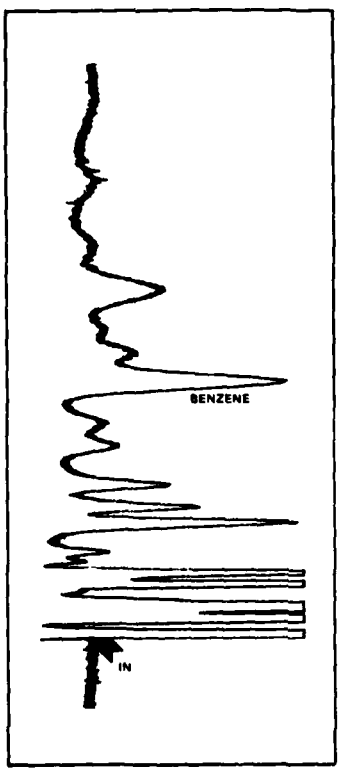

Fig.4 Results of Airscan measurement on a sample of typical suburban air containing benzene at approximately 2 parts-per-billion and employing an instrumental sensitivity factor of 5000. Early offscale peaks are gasoline derivatives of C_2 through C_5

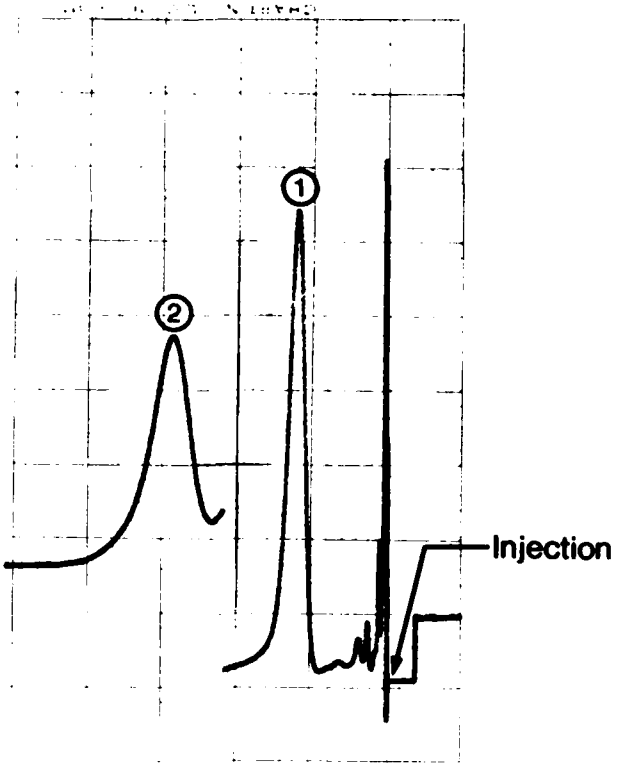

Fig.5 Airscan analysis of 0.25 ml α-pinene and limonene in air. Attenuation setting for α-pinene is 50 (Peak 1, 1.9 ppm, S = 5) and for limonene 5 (Peak 2, 620 ppb, S = 50). Column used was 10" x 1/16" 5% SE-30 on Chromosorb G 80/11. Chart speed was 0.5 cm/min and voltage span was 100mv

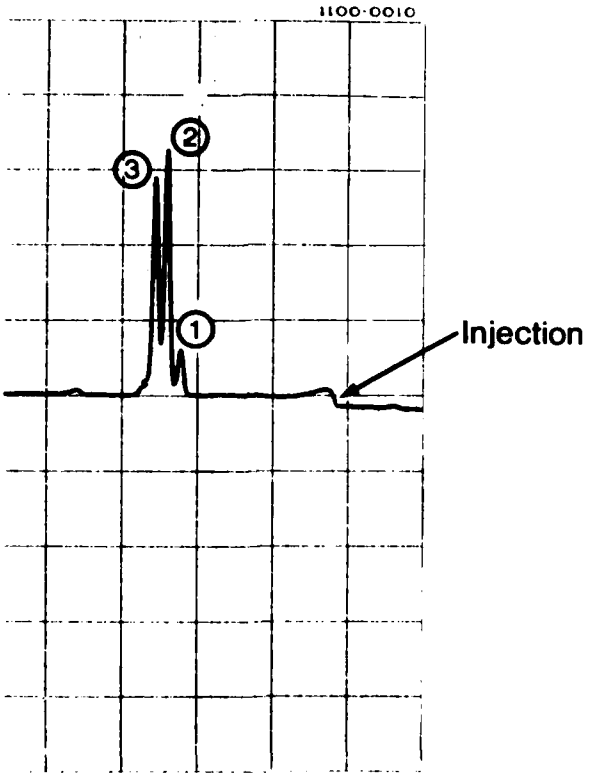

Fig.6 Airscan analysis of 30 μl arsine (1.8 ppm) and phosphine (3.3 ppm) in nitrogen. Attenuation setting was 5 and S was 6 for all peaks (Peak 1 = nitrogen, Peak 2 = phosphine and Peak 3 = arsine). Column used was 10" x 1/8" 5% SE-30 on Chromosorb G 80/100. Chart speed was 2 cm/min and voltage span was 100 mV

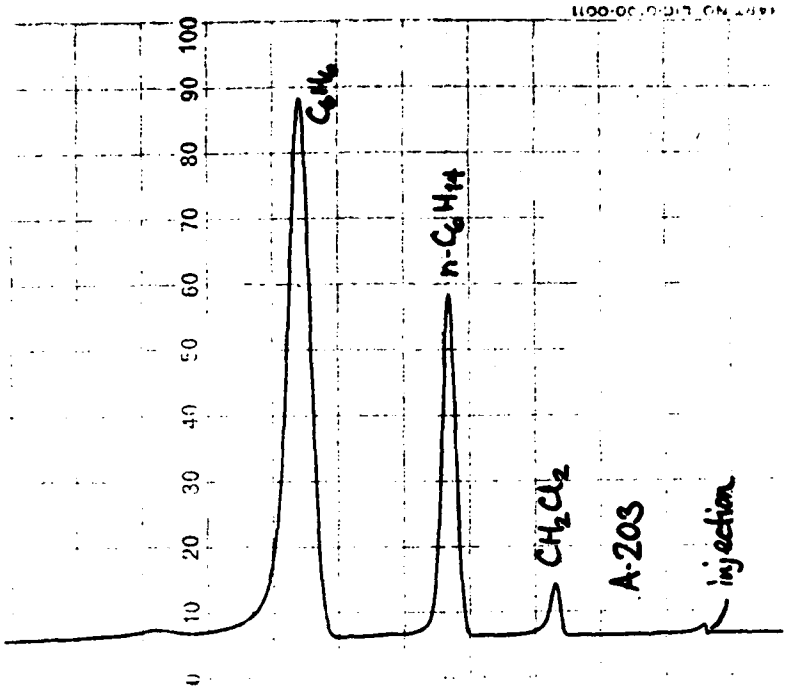

Fig.7 Airscan results showing relative response to air sample containing 10 ppm each of methylene chloride, n-hexane and benzene. Instrument sensitivity S = 0.5. Chart speed was 2 cm/min and voltage span was 100 mV

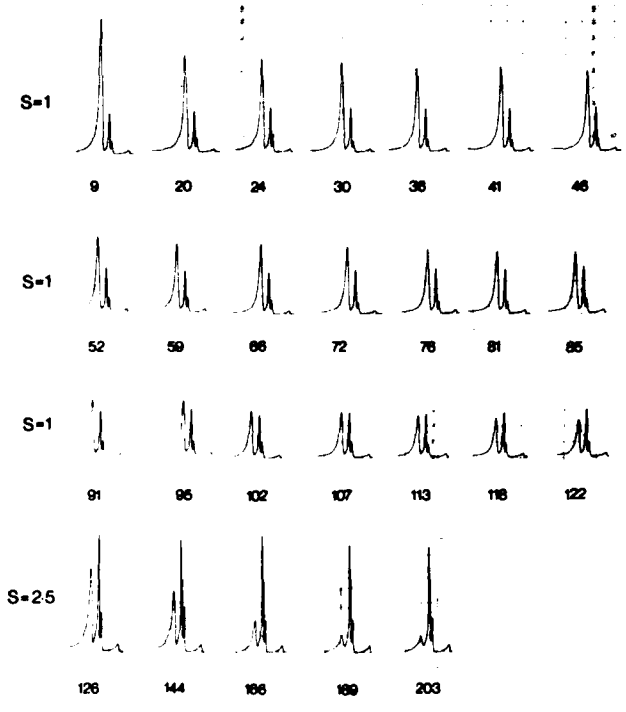

Fig.8 Results of Airscan measurement of sequential time-tracing of ethanol in the end-breath of a subject who had taken a dose of 0.3 gm/kg body mass. The numbers beneath each trace denotes the time in minutes after the ethanol consumption and the S factor denotes the instrument sensitivity. The ethanol peak is first on the left side in each trace

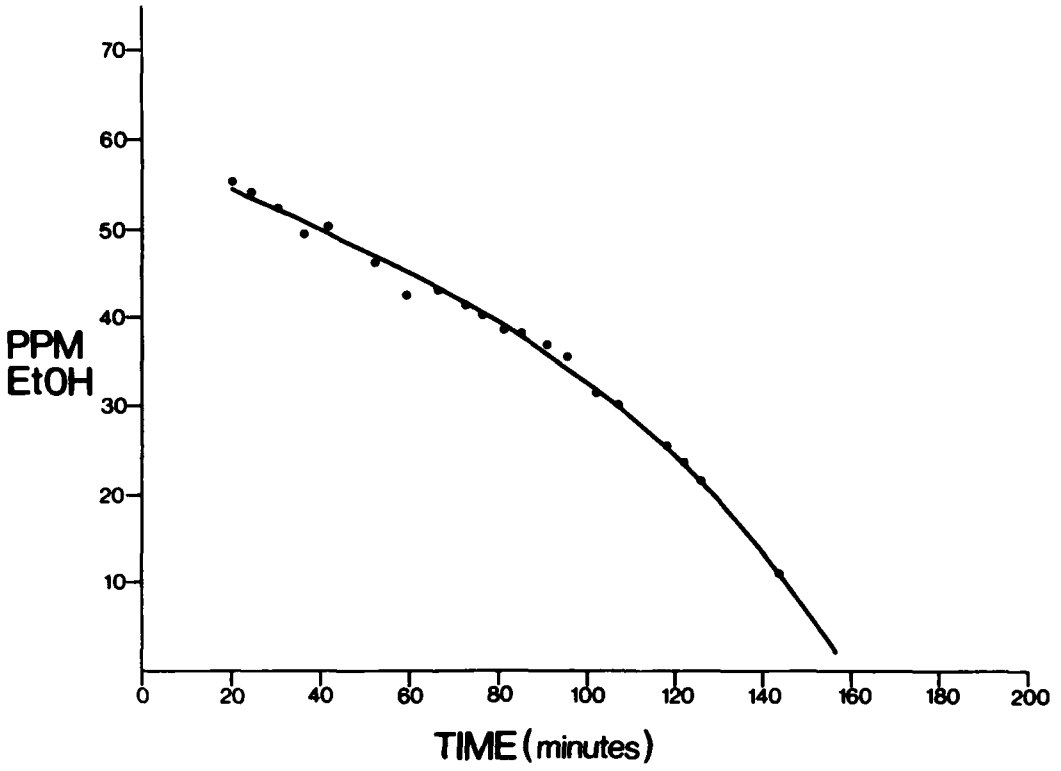

Fig.9 Plot of the same data shown in Figure 8 as a function of time, showing the presence of ethanol in the breath of this subject after imbibition

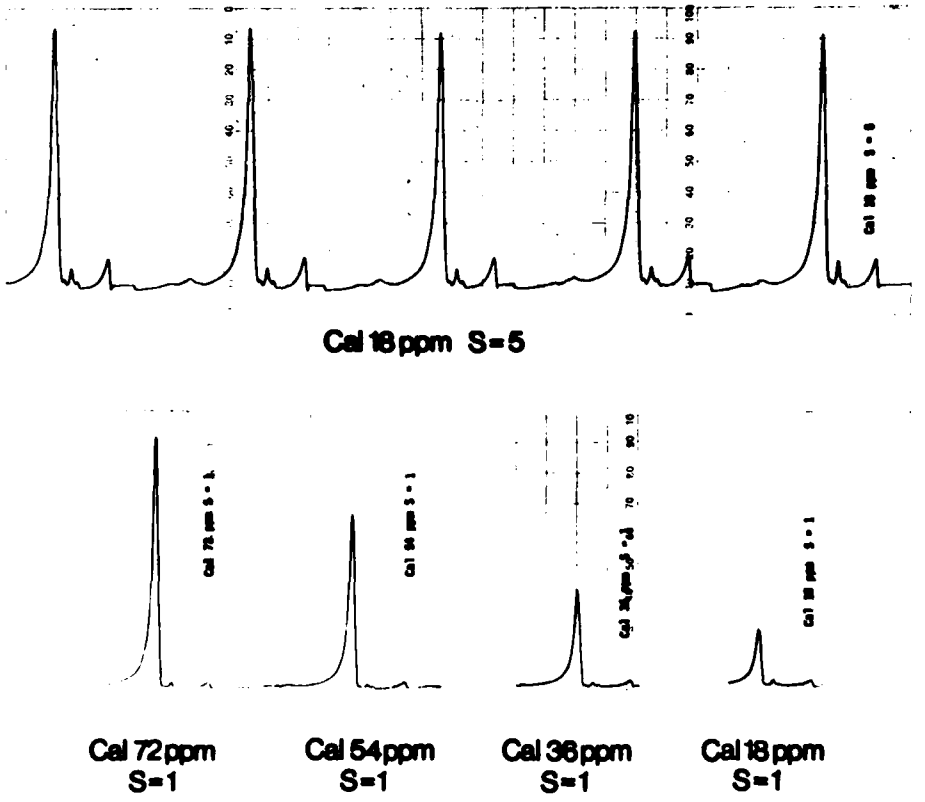

Fig.10 Airscan calibration measurements for ethanol at 18, 36, 52 and 72 ppm in air are shown on the bottom of this figure (S = 1). The top of the figure shows several calibrations of ethanol at 18 ppm in air (S = 5) from which the analytical precision for this compound was determined to be ± 1.5%

Fig.11 Airscan measurement of ethanol in the background air breathed by the subject described in Figure 9 along with an end-breath measurement from a subject who had taken no alcohol (S = 5)

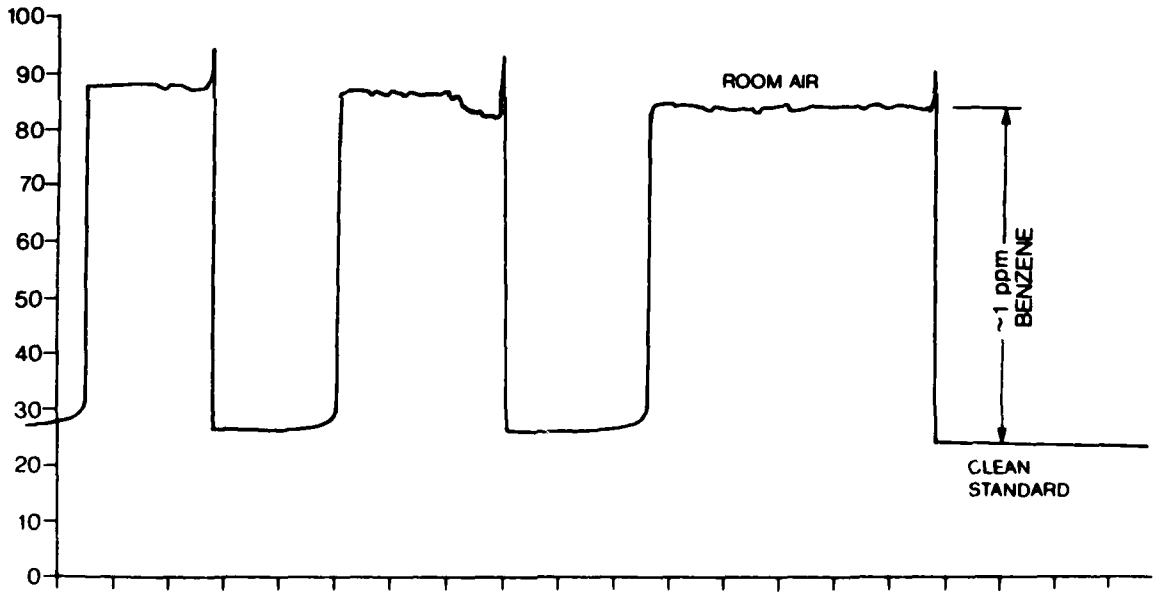

Fig.12 Results pertinent to Airscan instrument being used in the Total Ionizables Present (TIP) mode. The scan shows the difference between a clean air standard and an environment having a contamination comparable to 1 ppm benzene

```
PHOTOVAC    CCDA-3002
18:05:80    #      3

 ROM PACK #0102
 SE-30  5%
 CHROMOSORB G
 60/80   4' * 1/8''

 50.00 ml.
NR=4     PR=5     MR=8

01    UNKNOWN
     .34M  4.205 mVM

02    UNKNOWN
     .92M  44.62 mVM


03  TEST 1,2:1,0  18
    1.58M  85.30 mVM
 85.30 pg .0017ppm

04  TEST 2,2:1,0  15
    2.07M  32.52 mVM
 32.52 pg .0006ppm

05    UNKNOWN
    2.68M  360.3 mVM

06  CHLOROFORM    02
    3.27M  213.2 mVM
 5.267 ng .0212ppm

07  2,4-diMePENT  06
    3.97M  34.58 mVM
 115.9 pg .0005ppm

08    UNKNOWN
    4.62M  841.7 mVM


09  TEST 4,8:1,0  20
    5.92M  810.2 mVM
 810.2 pg .0162ppm

10  trCIETHYLENE  04
    6.75M  243.0 mVM
 396.0 pg .0014ppm
```

```
NUMBER    NAME

00  n-PENTANE
01  diCIMETHANE
02  CHLOROFORM
03  BENZENE
04  trCIETHYLENE
05  2-MePENTANE
06  2,4-diMePENT
07  3-MeHEXANE
08  n-HEPTANE
09  3-MeHEPTANE
10  MeCyHEXANE
11  234-trMePENT
12  2,4-diMeHEX
13  PRESS. SURGE
14  TEST 1,1:1,0
15  TEST 2,2:1,0
16  TEST 4,4:1,0
17  TEST 8,8:1,0
18  TEST 1,2:1,0
19  TEST 2,4:1,0
20  TEST 4,8:1,0
21  NITRIC OXIDE
22  PROPANE
23  isoBUTANE
24  n-BUTANE
25  2MeBUTANE
ENTER INJ. VOL. (ml)
```

Fig.13 Typical record from the Airscan data system

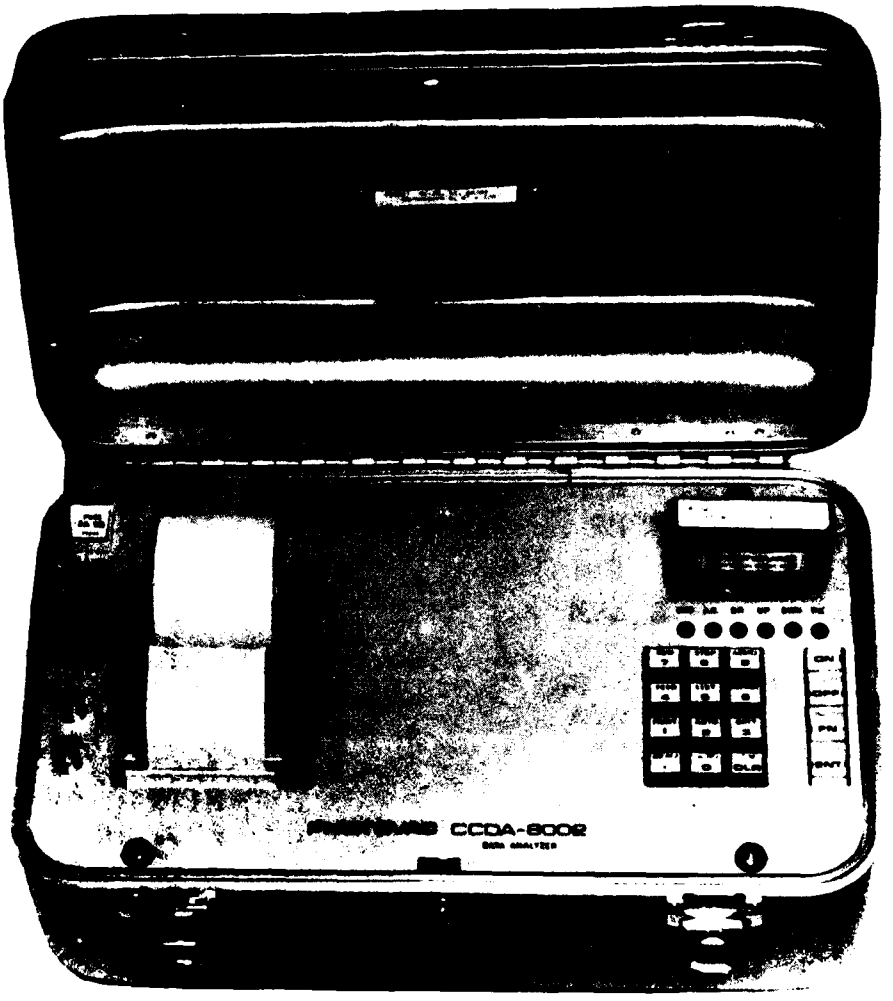

Fig.14 The Airscan data system

MODELE DE LABORATOIRE POUR EVALUER LA TOXICITE DES PRODUITS DE COMBUSTION

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L'évaluation de la toxicité des produits de dégradation thermique des matériaux doit pouvoir conduire à les classer envue de les sélectionner, en prenant en compte un certain nombre de critères. Nous avons essayé de définir une méthodologie relativement simple de screening par comparaison avec des explorations biologiques plus complexes. Un nouveau four permettant de mesurer des vitesses de combustion des matériaux est proposé. Les intoxications animales ont été réalisées chez le rat, soit laissé en ventilation spontanée, soit placé en ventilation assistée. Différents niveaux d'observation ont été choisis: DL 50, incapacitation, EEG, ECG, pression artérielle, lécithines et protéines alvéolaires. Pour l'analyse des résultats, la transformation de toutes les valeurs brutes en leurs valeurs centrées réduites et le calcul d'un indice de PEARSON modifié permettent d'aborder le classement des matériaux par un index global.

INTRODUCTION.

L'évaluation de la toxicité des produits de thermolyse des matériaux est très préoccupante et le choix de l'utilisation de ces derniers nécessite de pouvoir les classer en fonction des risques qu'ils peuvent entraîner et cela impose:

. la codification d'une méthode prenant en compte les facteurs principaux que l'on doit définir en fonction des exigences de la routine dont les mailles ne doivent pas être trop larges pour assurer une couverture suffisante. Le choix des paramètres doit être en accord avec les risques maximums présentés par un matériau placé dans un scenario défini.

. le moyen de juger de l'ensemble des modifications des variables par une approche multivariée.

L'évaluation toxicologique repose sur le problème f ondamental de l'établissement de relations doses-effets. La dose dépend du modèle feu choisi, donc du scenario proposé, qui ne doit pas être trop éloigné d'une certaine réalité. Elle dépend aussi du mode d'administration des atmosphères toxiques aux animaux

laissés en ventilation spontanée (VS) ou placés sous ventilation contrôlée(VC) avec les avantages et les inconvénients que cela comporte. Dans le premier cas, on estime la toxicité globale des atmosphères, dans le scond la toxicité au niveau de l'alvéole pulmonaire et ses conséquences. L'effet demande de choisir des critères de dérèglement du matériel biologique pour apprécier les réponses. Ces critères sont de plusieurs natures et on doit connaître leurs significations exactes afin de ne pas les mélanger de façon arbitraire ou inconsidérée lors des interprétations. On peut ainsi définir les significations:

- paramètres de fonctionnement des organes:

. létaux: c'est d'abord la mort quise caractérise par l'arrêt d'un ou plusieurs organes vitaux (ventilation, coeur, système nerveux); son apparition est généralement constatée par la perte de la stature et des réflexes et l'arrêt respiratoire; on peut aussi la définir plus précisément par l'arrêt de l'ac tivité cérébrale (EEG) ou cardiaque (ECG)

. sublétaux: c'est par exemple l'incapacitation; l'absence d'activité motrice présente un risque grave.

- paramètres de disfonctionnement biologique:ils sont essentiellement de signification sublétale, comme la mesure des gaz du sang, l'étude de la chimie pulmonaire(surfactant).

Le phénomène définitf de la mort rapide n'apporte pas énormément de renseignements(DL50), bien que permettant un premier tri. Il semble plus judicieux de rester dans une phase sublétale(diminution 50% d'activité d'un organe vital) afin de tolérer une récupération éventuelle permettant des jugements plus fins. En fait, il vaut mieux considérer les possibilités de survie que les conditions de mort dans une intoxication.

La connaissance analytique des toxiques majeurs (CO, HCl ou HCN) est intéressante car un toxique majeur peut être représentatif de toute la toxicité; elle montre la cinétique de thermolyse par son émission et peut servir de référence; elle permet d'étalonner les mesures de toxicité par un toxique isolé connu. Mais elle est insuffisante car un toxique majeur est assez rarement représentatif de toute la toxicité potentielle d'un matériau; les associations de toxiques(synergies,antagonismes) ne sont pas prises en compte; des "supertoxiques",type acroléine, ne sont pas forcément considérés; une analyse même approfondie n'est pas obligatoirement complète. Le dosage des toxiques majeurs est une démarche analytique, la réponse biologique est d'ordre synthétique. On peut quand même dire que, si dans les gaz de combustion un toxique majeur atteint des valeurs reconnues comme mortelles l'essai animal est inutile. Dans le cas contraire, l'utilisation d'animaux est indispensable.

Dans une approche multivariée, l'appréciation de risques combinés a été faite par les Américains sous forme d'un "Combined Hazard Index"(CHI) prenant en compte trois critères (fumée, température, toxicité). La valeur commune à ces trois variables est le temps au bout duquel l'une d'entre elles prend la valeur maximum.

Nous avons abordé le problème de la manière suivante:

. sur le plan du modèle feu, la température de thermolyse de 500°C a été choisie, assez représentative des feux qui couvent; c'est souvent là que le dégagement de toxiques est le plus important. La vitesse de combustion du matériau est capitale à connaître, le développement d'un feu étant très lié au temps et à la masse de matière engagée. Les gaz toxiques majeurs doivent être dosés en continu (référence).

. sur le plan des modèles animaux, nous avons retenu les rats et les souris, intoxiqués soit en ventilation spontanée (toxicité globale), en calculant les DL50 (g de matériau brûlé par m^3 d'air) et en mesurant les temps d'incapacitation(activité motrice spontanée appréciée par sismographie) soit en ventilation contrôlée (toxicité alvéolaire) après intubation trachéale et assistance ventilatoire par pompe.

. nous avons cherché à prendre en compte le maximum de variables et à en juger de façon multivariée par leur transformation en valeurs centrées réduites puis estimation des significations par une mofification du test de PEARSON (χ^2) conduisant à un classement des matériaux.

METHODOLOGIE.

1. Matériaux.

Les atmosphères contenant des toxiques majeurs ont été préparées à partir d'obus de gaz purs dilués avec de l'air dans des ballons en SARAN. Le bois est du peuplier, le PVC un polychlorure de vinyle rigide, les deux mousses de polyuréthanes, l'une souple non ignifugée(PUS), l'autre rigide ignifugée (PUR-ig).

2. Modèles feu.

Deux types de four ont été utilisés, un four mobile non asservi(FM), un four asservi (FA).

2.1. Four mobile non asservi:

Un four circulaire se déplace le long d'une nacelle en quartz contenant des échantillons sous forme de réglettes. Le débit d'air est de 200l/h, la vitesse du four de 1 cm/mn. L'air et le four se déplacent dans le même sens(condition co-courant), brûlant l'échantillon au fur et à mesure.

2.2. Four asservi:

Dans ce cas, un chariot porte-échantillon est mobile et engage le matériau dans un four circulaire fixe à vitesse variable(figure n°1). Deux thermocouples sont placés au niveau du four, l'un (T1) servant de témoin à la sortie, l'autre (T2) à l'entrée du four au voisinage de l'échantillon. Quand le matériau est

introduit dans le four, la température T2 s'élève par rapport à la température T1 témoin imposée au four car des calories sont libérées lors de la dégradation thermique du matériau; la marche du chariot est alors arrêtée. Quand la dégradation est terminée, les températures T1 et T2 s'égalisent et le chariot se remet en marche, nourrissant le feu. L'activité du moteur entraînant le chariot est donc en relation directe avec la vitesse de combustion dudit matériau. Les échantillons sont également sous forme de baguettes et le débit d'air traversant la chambre est de 130l/h.

2.3. Dosage des toxiques majeurs:

A la sortie de la chambre, une partir aliquote des gaz est prélevée et le CO est dosé par spectrophotométrie IR(appareil COSMA), HCN et HCl par potentiométrie (appareil RADIOMETER) en fonction du type de matériau

3. Modèles animaux.

3.1. Ventilation spontanée:

- DL50: les rats de souche Wistar, d'un poids moyen de 220 g, sont placés dans 5 tubes à contention reliés à une boule répartitrice des gaz, elle-même reliée à la sortie des fours. L'augmentation des doses est assurée par augmentation des poids d'échantillons et la DL50 est calculée par la méthode de REED & MUNCH dite des totaux cumulatifs.

- Activité motrice spontanée: un sismographe simple a été mis au point (figure n°1). Sur un portoir de burette, une forte lame de scie est fixée et supporte à son extrémité libre une chambre en verre de 1 l., perpendiculaire dans le plan, pouvant contenir un rat ou deux souris. L'extrémité libre de la lame est reliée par un fil à l'axe d'un petit moteur synchrone dont les bornes sont reliées à un enregistreur. La lame de scie branle en fonction des mouvements des animaux et notamment de leur activité exploratoire, ce qui fait tourner l'axe du moteur qui développe un courant que l'on enregistre. La sensibilité de ce système est grande et dans le cas des rats, la ventilation peut être enregistrée. L'activité de souris (Swiss d'un poids moyen de 25g) exposées à un balayage d'air pur dure plus de deux heures, ce qui est suffisant pour la durée de nos expériences. Lors d'intoxications, deux mesures sont effectuées: le délai d'incapacitation (temps au bout duquel l'activité des animaux cesse) et la durée d'incapacitation (temps au bout duquel l'activité est reprise.

3.2. Ventilation contrôlée.

Elle consiste à établir le "Physiogramme" (figure n°2). Une trachéotomie est pratiquée chez le rat sous anesthésie flash par l'éther éthylique. L'animal est ensuite placé sous ventilation artificielle avec une pompe BIRD (débit d'air 20 l/h) et immédiatement curarisé avec 0,75 ml de flaxédil(triiodométhylate de gallamine) administré par voie intra-péritonéale. Une bonne anesthésie locale est alors pratiquée avec la procaïne; la carotide est canulée et un robinet à double voie permet de faire des prélèvements de sang artériel sur le trajet de la prise de pression artérielle. Trois échantillons de sang sont pris au cours d'une intoxication, le premier en témoin avant l'intoxication, le second à la fin de cette dernière (30mn) le dernier après une période de récupération(120mn). L'EEG(bioccipital) et l'ECG(dérivation D1) sont enregistrés pendant toute la durée de l'expérience. Les gaz du sang (PO_2 et PCO_2), le pH artériels sont mesurés par l'appreil RADIOMETER.

Le Physiogramme est un diagramme à 3 coordonnées. En abscisse l'activité du système nerveux central est exprimée en fréquence de l'EEG. En ordonnées, sont reportés le pouls d'un côté et la pression artérielle de l'autre. L'état de l'animal à un moment donné est donc représenté par un point. Les déplacements de ce

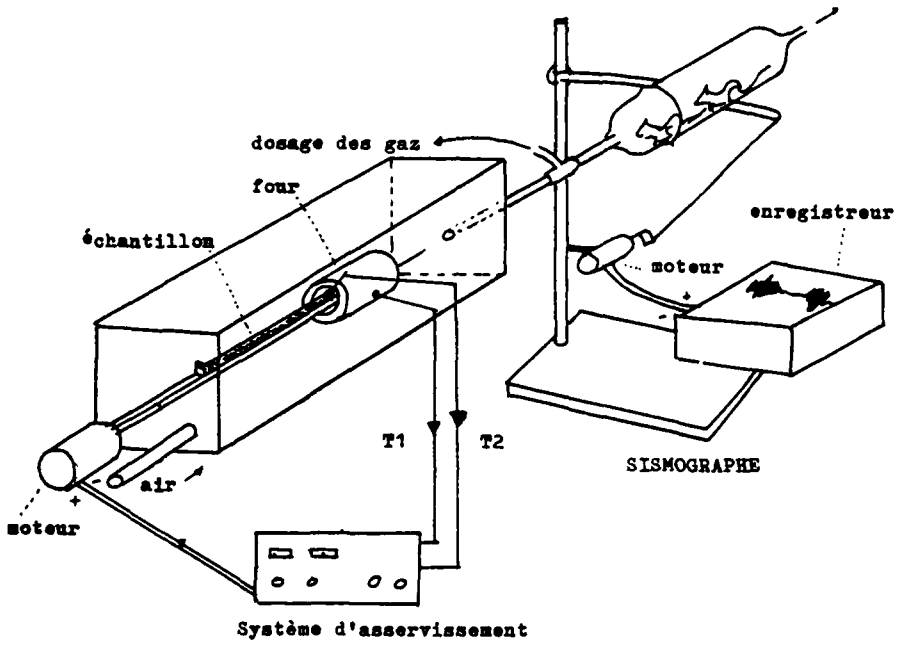
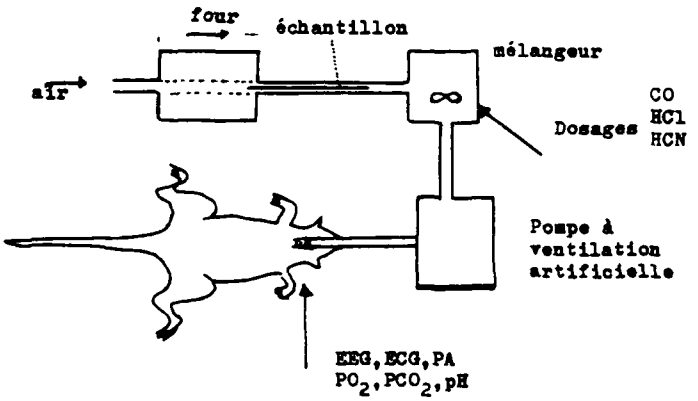


Figure n°1: Schema du four asservi et du sismographe mesurant l'activité spontanée des souris

T1=thermocouple de référence - T2=thermocouple placé au niveau du matériau



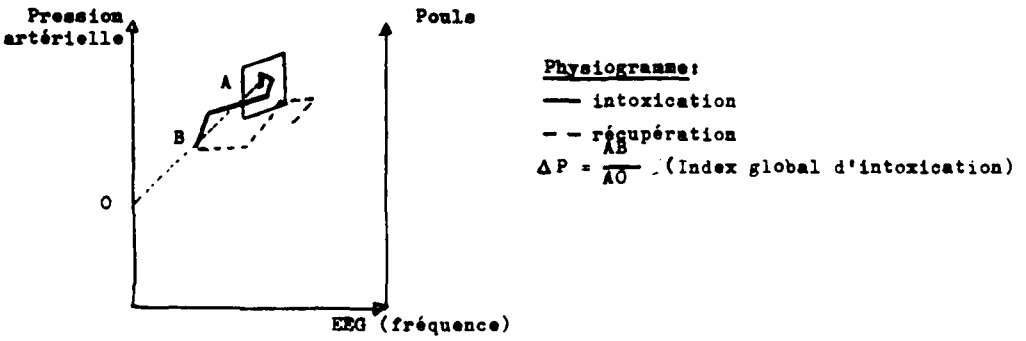


Figure n°2: Schema d'intoxication du rat en ventilation contrôlée (physiogramme) et établissement du diagramme; thermolyse du matériau par le four mobile (FM).

dernier permettent de suivre facilement les évolutions de l'intoxication et de la récupération. On peut également calculer à partir de ce diagramme un "Index global d'intoxication" dit $\Delta P\%$. Si, comme dans la figure n°2, le point significatif de l'état du rat va de A à B par exemple, on peut prolonger AB jusqu'à son intersection O avec une corrdonnée. Le rapport des distances AB/AO donne le pourcentage de dégradation de l'état de l'animal, soit le $\Delta P\%$. On peut ainsi obtenir des courbes dose-réponse.

L'exploration du revêtement alvéolaire a été fait par lavage des poumons avec une solution saline isotonique puis dosage des protéines par le réactif de LOWRY et des lécithines par chromatographie en couche mince quantitative (PRE et col. (1) .

4. Analyse des résultats.

4.1. Valeurs centrées réduites:

Il faut comparer les variations à l'intérieur d'une variable et dans différentes variables exprimées dans des unités différentes. Il faut faire d'une part abstraction de ces unités et d'autre part garder des dispersions comparables par rapport au témoin dans chaque variable. A ce moment là, tous les chiffres deviendront comparables. Le moyen est de transformer chaque valeur brute x en sa valeur centrée réduite $\frac{x - \bar{x}}{s}$, $\bar{x}$ correspondant à la moyenne des valeurs obtenues dans la variable et s l'écart-type. On peut alors faire un bilan des variations de plusieurs variables.

4.2. Classement des composés et des matériaux:

Nous avons alors utilisé le principe du test de PEARSON (χ^2) pour classer les composés par rapport aux témoins. L'application est bonne pour les variations des valeurs biologiques telles que 1=EEG, 2=ECG, 3=PA, 4=PO_2, 5=PCO_2 et 6=pH. Si pour chaque variable, la valeur témoin est t1 et la valeur de l'essai e1, on peut écrire:

$$\chi^2 = \frac{(e1-t1)^2}{t1} + \frac{(e2-t2)^2}{t2} + \ldots \frac{(en-tn)^2}{tn}$$

Plus cette valeur est élevée, plus le composé dérègle le témoin et un classement prenant en compte les diverses variables mesurées peut être obtenu.

RESULTATS.

1. Modèles feux.

A la température de 500°C, l'activité du moteur qui repart ou s'arrête en fonction de la combustion est correcte pour tous les matériaux sauf pour le PVC. Dans ce dernier cas, le matériau fond en effet sans brûler, mais subit pourtant une dégradation thermique; le moteur introduit constamment du matériau neuf dans la source de chaleur. Une première distinction peut se faire par la mesure des vitesses de combustion, exprimées en mg consommé par gramme de matériau et par minute ($mg.g^{-1}.mn^{-1}$). Ainsi, le PVC nourrit constamment le feu, la vitesse de combustion de la mousse souple de PU est plus grande que celle de la mousse rigide ignifugée, toutes deux étant supérieures à celle du bois. Cela reflète une certaine réalité. On peut constater par ailleurs que le four mobile non asservi ne respecte pas cette caractéristique des matériaux (Tableau n°I).

Un deuxième comportement peut être apprécié par la production du toxique majeur, exprimée en ppm de toxique

émis par mg de matériau et par litre d'air mis en jeu ($ppm.mg^{-1}.l^{-1}$). La comparaison de l'action des deux types de fours conduit aux constatations suivantes:

Dans le cas du CO, quel que soit le matériau, les écart-types sont relativement importants, montrant que la production de ce gaz n'est pas très reproductible, quel que soit le four employé. Elles restent toutefois du même ordre de grandeur et ne sont pas significativement différentes quel que soit le mode de thermolyse choisi, sauf dans le cas du PVC. Dans ce dernier cas, la production d'HCl est pourtant identique dans les deux modes de dégradation, montrant bien le danger que ce matériau présente simplement en fondant. Il est intéressant de noter que les polyuréthanes sont de bons fournisseurs de CO, spécialement avec le four asservi. Si, avec les deux fours, le PUS fournit les mêmes concentrations d'HCN, ce n'est pas le cas avec le PUR-ig produisant plus de toxique dans le four mobile.

Matériau / Critère		Peuplier	PVC	PUS	PUR-ig
vitesse de combustion $mg.g^{-1}.mn^{-1}$	FM	16,6 ± 0,5	16,6 ± 0,5	16,6 ± 0,5	16,6 ± 0,5
	FA	36,5 ± 6	136 ± 3	112 ± 17	94 ± 5
CO $ppm.mg^{-1}.l^{-1}$	FM	140 ± 21	116 ± 46	92 ± 35	107 ± 65
	FA	103 ± 21	30 ± 9	126 ± 37	177 ± 30
HCl $ppm.mg^{-1}.l^{-1}$	FM		250 ± 6		
	FA		273 ± 9		
HCN $ppm/mg^{-1}.l^{-1}$	FM			5,3 ± 1,3	19,7 ± 4,5
	FA			7,5 ± 1,4	8,3 ± 3

Tableau n°I: Vitesses de combustion et production de gaz toxiques par divers matériaux en fonction des fours employés pour leur dégradation (FM= four mobile - FA= four asservi).

2. Effets biologiques.

2.1. Critères léteux.

C'est essentiellement la détermination des DL50 en ventilation spontanée comme en ventilation contrôlée (VS et VC), les animaux étant exposés au four mobile pendant 30 minutes. Le rapport des valeurs VS/VC permet d'apprécier le risque alvéolaire dû aux gaz et vapeurs; s'il est inférieur à 1 la défaillance ventilatoire est supprimée par la ventilation artificielle mais la tolérance alvéolaire est bonne, c'est l e cas de CO. On possède alors un argument thérapeutique favorable. Si le rapport est supérieur à 1, le risque alvéolaire est grand quand le toxique peut atteindre ce niveau, c'est le cas d'HCl.

Dans le cas du bois, le CO explique bien la toxicité observée, ce qui n'est pas vu pour les PU et le PVC. D'autres toxiques doivent alors être pris en considération (Tableau n°II).

2.2. Critères sublétaux.

. Incapacitation: ces essais ont été menés avec des gaz de thermolyse produits par le four asservi sur les souris. Nous avons conservé pour le délai d'apparition de l'incapacitation le sigle Ti (time to incapacitation). Le Tableau n°III montre que cette valeur est plus facile à mesurer que la d urée de l'

incapacitation qui peut être très longue. Mais Ti sera extrêmement long chez le témoin et donc difficile à manipuler. Par contre, 1/Ti est à l'inverse très court; chez les animaux exposés plus Ti sera petit, plus 1/Ti sera grand et l'incapacitation forte.

	Ventilation spontanée VS	Ventilation contrôlée VC	Rapport VS/VC
CO ppm	4100	10000	0,41
HCl ppm	20000	5500	3,63
HCN ppm	820	380	2,15
Peuplier g/m^3	37,5 CO = 4300	55,2 CO = 8800	0,68
PVC g/m^3	28,0 CO = 1000 HCl = 8000	15,0 CO = 400 HCl = 4000	1,86
PUS g/m^3	26,0 CO = 3300 HCN = 150	17,5 CO = 2200 HCN = 130	1,48
PUR-ig g/m^3	5,5 CO = 500 HCN = 140	7,2 CO = 1270 HCN = 65	0,76

Tableau n°II : DL50 des toxiques majeurs et des matériaux leur donnant naissance pour des intoxications de 30 mn chez le rat placé en ventilation contrôlée ou laissé en ventilation spontanée.

Une deuxième approche a consisté à rechercher la concentration de produit brûlé dans les atmosphères (en g/m^3) provoquant un Ti de 10 minutes. Ainsi un terme de comparaison peut être trouvé entre les différents matériaux. Le PUR-ig est le plus incapacitant, suivi du PUS puis le bois et le PVC paraissent nettement moins dangereux. Le CO est assez bien représentatif de la toxicité du bois, mais les produits de thermolyse des PU sont plus toxiques que l'HCN qu'ils génèrent. Le cas d'HCl est difficile à interpréter car ce gaz est facilement fixé par le corps des animaux et le matériel de la chambre d'exposition, surtout en présence d'un fort degré hygrométrique, comme c'est généralement le cas. La concentration qui incapacite les animaux semble être de 8000 ppm, très supérieure à celle induite par les produits de thermolyse du PVC dans lesquels le CO doit prendre sa part. Mais en fait, la toxicité de ce genre de composé s'exprime tardivement par un oedème aigu pulmonaire et le Ti n'est pas un bontémoin de ce genre de pathologie.

Tableau n°III	Ti (10 minutes) conc.toxique	Durée de l'incapacitation	CO ppm	HCL ppm	HCNppm
CO	5600 ppm	35 min.	5600		
Peuplier	48 g/m^3	60	5000		
HCl	8000 ppm	mort possible		8000	
PVC	69 g/m^3	45	3200	3400	
HCN	205 ppm	40			205
PUS	27 g/m^3	17	3800		120
PUR-ig	21,5 g/m^3	15	4000		155

. Index d'intoxication en ventilation contrôlée (ΔP_{fg}): il permet d'apprécier la toxicité des gaz et vapeurs administrés par trachéotomie au niveau alvéolaire. Le four mobile a servi de générateur de produits de thermolyse. A partir des Physiogrammes, on peut dresser des courbes exprimant le ΔP en fonction du temps. A titre d'exemple, la figure n°3 fournit les courbes obtenues avec les toxiques majeurs. On constate que la récupération parait possible jusqu'à 5000 ppm pour le CO, qu'elle est moins bonne mais encore acceptable pour HCN jusqu'à 220 ppm, très mauvaise pour HCl à partir de 6000 ppm. Pendant l'intoxication, à l'inverse, les désordres sont grands sous CO et HCN, faibles sous HCl.

On peut aussi porter les valeurs du $\Delta P\%$ en fonction de concentrations croissantes de CO et HCN à 30 minutes et d'HCl à 120 minutes car pour ce dernier les phénomènes toxiques apparaissent plus tard. Si l'évolution est progressive pour le CO, il y a une articulation nette pour HCl à 5000 ppm et pour HCN à 210 ppm. De là on a pu calculer les DL50 des matériaux en ventilation contrôlée et l'on peut définir une valeur sublétale, la diminution de 50% du ΔP (dite $\Delta P50$). Des calculs du même ordre ont été faits pour les toxiques majeurs et les matériaux choisis et les résultats sont les suivants:

P50 pour CO = 3000ppm, HCN = 250 ppm, HCl = 5000 ppm, Peuplier = 50 g/m^3, PVC = 12 g/m^3, PUS = 27 g/m^3, PUR-ig = 21,5 g/m^3.

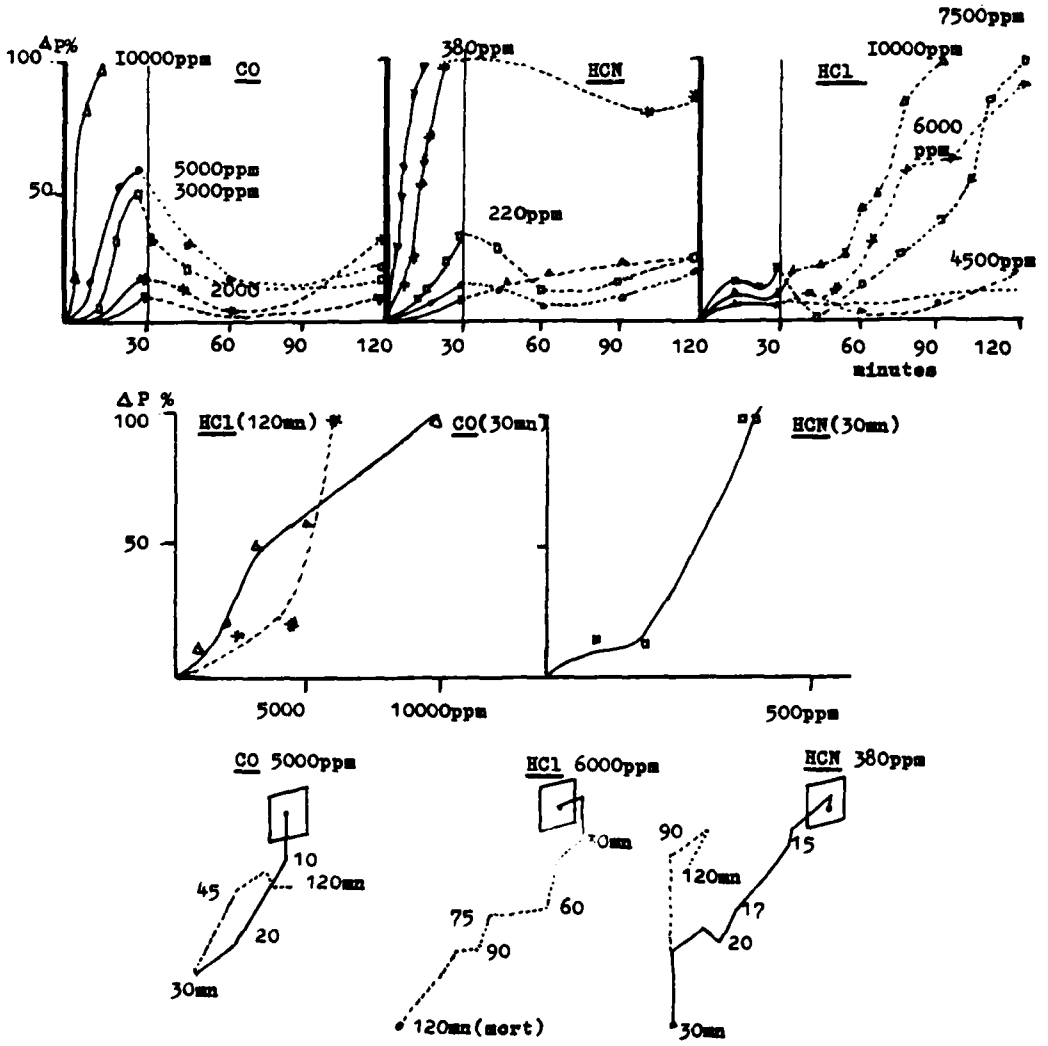


Figure n°3: Index d'intoxication ($\Delta P\%$) en fonction du temps ou des doses de toxique à partir des physiogrammes déterminés chez le rat, en ventilation contrôlée.

2.3. Interprétation.

Nous allons donc maintenant tenter d'utiliser les résultats obtenus jusqu'à présent pour obtenir un classement objectif des matériaux en reprenant les valeurs respectives mesurées dans les 5 critères suivants: (I) = vitesse de combustion - (II) = DL50 en VS - (III) = DL50 en VC - (IV) = ΔP50 - (V) = Ti 10 min. En transformant les valeurs brutes en leurs valeurs centrées réduites, on considère que les variations dans chaque variable ont le même poids, mais on a admis que les paramètres choisis étaient d'égale importance, et les écarts observés seront en accord avec les risques respectifs. Puisque les valeurs les plus fortes sont les moins dangereuses, il faut changer le signe des vitesses de combustion dont les risques sont d'autant plus grands qu'elles sont élevées. Nous adopterons donc la séquence suivante: -(I)+(II)+(III)+(IV)+(V). La somme de tous ces écarts sera donc représentative de l'ensemble des variations enregistrées pour chaque critère. Le Tableau n°IV résume ces données. Le classement obtenu est le suivant, dans l'ordre croissant de toxicité: Peuplier - PVC - PUS - PUR-ig.

Matériau	Vitesse de combustion (I)	DL 50 VS (II)	DL 50 VC (III)	P 50 VC (IV)	Ti(10mn) (V)	
VALEURS BRUTES	$mg.g^{-1}.mn^{-1}$	g/m^3	g/m^3	g/m^3	g/m^3	
Peuplier	38,5	37,5	55,2	50	48	
PVC	136	28	15	12	69	
PUS	119	26	17,5	17	27	
PUR-ig	94	5,5	7,2	7	21,5	
$\bar{m}$	96,4	24,2	23,7	21,5	41,3	
s	43,4	13,5	21,4	19,4	21,7	
VALEURS CENTREES REDUITES	-(I)	+(II)	+(III)	+(IV)	+(V)	$\sum$
Peuplier	+ 1,38	+ 0,98	+ 1,47	+ 1,47	+ 0,31	+ 5,61
PVC	- 0,91	+ 0,28	- 0,40	- 0,50	+ 1,27	- 0,26
PUS	- 0,52	+ 0,13	- 0,30	- 0,23	- 0,66	- 1,58
PUR-ig	+ 0,05	- 1,38	- 0,77	- 0,75	- 0,91	- 3,76

Tableau n°IV: Classement des matériaux par addition des valeurs centrées réduites des variations obtenues dans 5 variables fondamentales sélectionnées pour juger de risques dûs à des matériaux.

3, Comparaison approfondie de deux polyuréthanes.

Nous venons de voir que les critères retenus précédemment séparaient bien des matériaux différents. En mettant en jeu un nombre plus important de variables notamment biochimiques, nous allons augmenter le pouvoir de discrimination de la méthode employant les valeurs centrées réduites en comparant les deux polyuréthanes choisis (PUS et PUR-ig) à leur toxique majeur HCN. Nous avons utilisé les résultats obtenus lors d'intoxications des rats en ventilation contrôlée servant à la détermination des Physiogrammes. Le PUS (17 g/m^3) et le PUR-ig (7g/m^3) ont été brûlés dans le four mobile et leurs produits de combustion comparés à l'effet d'une atmosphère contenant 150 ppm d'HCN, concentration maximale de ce toxique majeur que ces derniers pouvaient contenir.

Les variables prises en compte peuvent être rattachées à plusieurs compartiments. Les atmosphères toxiques pénètrent au niveau pulmonaire où deux variables sont mesurées, les lécithines (L) et les protéines (P) des liquides de lavage alvéolaire. Les toxiques pénètrent ensuite dans le sang, deuxième compartiment, où sont estimées trois variables, la PO_2, la PCO_2 et le pH. Ils agissent enfin au niveau des compartiments cellulaires où trois variables sont retenues, la fréquence de l'EEG, le pouls(ECG) et la pression artérielle (PA). Le Tableau n°V réunit les valeurs brutes et leurs valeurs centrées réduites correspondantes.

Variables	L	P	L/P	PO_2	PCO_2	pH	EEG	ECG	PA
Témoins	71	165	0,43	87,3	42,3	7,40	17,3	389	142
HCN 150 ppm	71	386	0,18	100,3	43,6	7,32	17	360	148
PUS 17 g/m^3 (HCN = 110 ppm)	72	440	0,16	59,9	40,6	7,13	12	260	87
PUR-ig 7 g/m^3	120	840	0,14	105,4	38,7	7,27	9,3	200	107
$\bar{m}$	83,5	458		88,2	41,3	7,28	13,9	302	121
s	24,3	281		20,3	2,12	0,11	3,9	88	29
VALEURS CENTREES REDUITES									
Témoins	- 0,51	- 1,04		- 0,04	+ 0,47	+ 1,09	+ 0,82	+ 0,99	+ 0,72
HCN	- 0,51	- 0,25		+ 0,59	+ 1,08	+ 0,36	+ 0,79	+ 0,66	+ 0,93
PUS	- 0,47	- 0,06		- 1,39	- 0,33	- 1,36	- 0,49	- 0,48	- 1,17
PUR-ig	+ 1,5	+ 1,36		+ 0,84	- 1,22	- 0,09	- 1,18	- 1,16	- 0,48

Tableau n°V: Moyennes des valeurs brutes des variables enregistrées à 30 minutes chez les rats en ventilation contrôlée int oxiqués par HCN et les produits de thermolyse de 2 polyuréthanes et transformations en valeurs centrées réduites.

C'est alors que nous pouvons nous inspirer de l'indice de PEARSON, dit test du χ^2. Il nous permet de chiffrer les écarts pour les différents produits en comparant les variations des valeurs centrées réduites sous toxique dans chaque variable par rapport au témoin et en faisant la somme. Le Tableau n°VI explicite les résultats. On s'apperçoit que les deux polyuréthanes sont beaucoup plus dangereux que l'HCN qu'ils génèrent. La comparaison des deux matériaux entre eux est pour l'instant difficile à faire par unité de g/m^3 mis en jeu car le test de χ^2 n'est pas linéaire et il n'est pas acceptable de diviser l'indice global obtenu par le nombre de g/m^3 utilisés. Il faudra établir une table personnelle de ces indices pour autoriser une telle comparaison. Toutefois, le PUS (17 g/m^3) parait moins dangereux que le PUR-ig (7 g/m^3). Dans cet esprit, deux initiatives peuvent être prises, soit de comparer une série de matériaux à g/m^3 identiques, soit définir les g/m^3 acceptables à χ^2 égaux.

Une deuxième considération est importante. Les indices partiels obtenus dans chaque compartiment nous montrent que pourdes effets physiologiques comparables (7,19 pour le PUS et 9,01 pour le PUR-ig), la mousse rigide provoque de grandes perturbations au niveau des poumons (indice 13,45), ce qui est un mauvais pronostic comparée à la mousse souple (indice 0,92). Par contre, cette dernière entraîne davantage de perturbations sanguines (indice 52,42 au lieu de 26,7). De plus, en examinant les indices obtenus pour chaque variable, on voit très clairement les liaisons qui peuvent exister entre les différentes perturbations. Dans le cas d'HCN, seule la PO_2 est significativement modifiée. On connait bien l'action inhibitrice de ce toxique sur la consommation d'oxygène cellulaire.

Dans le cas du PUS, le revêtement alvéolaire n'est pratiquement pas altéré. Par contre, la PO_2 est très affectée et le pH en subit les conséquences. La répercussion physiologique principale se fait alors sur la pression artérielle. Dans le cas du PUR-ig, l'altération pulmonaire est flagrante. L'équilibre PO_2/PCO_2 est significativement perturbé, en accord avec l'atteinte alvéolaire qui dérègle les échanges gazeux. Les conséquences physiologiques, surtout centrales, sont nettes.

	INDICES PAR VARIABLE (calculés sur les valeurs centrées réduites)								$\Sigma = \chi^2$
	L	P	PO_2	PCO_2	pH	EEG	ECG	PA	Indice global
HCN	0	0,6	9,92	0,79	0,49	0,007	2,75	0,06	14,62
PUS	0,003	0,92	45,56	1,36	5,5	2,12	0,11	4,96	80,53
PUR-ig	7,92	5,53	19,36	6,07	1,27	4,83	2,18	2,0	49,16
	INDICES PARTIELS								
	Poumons Σ (L+P)		Sang Σ (PO_2 + PCO_2 +pH)			Organes Σ (EEG + ECG + PA)			
HCN	0,6		11,2			2,82			
PUS	0,92		52,42			7,19			
PUR-ig	13,45		26,7			9,01			

Tableau n°VI: Calculs des écarts sur le principe de l'indice de PEARSON (test du χ^2) entre HCN et les deux mousses de Polyuréthanes par rapport aux témoins en fonction de différentes variables biologiques.

CONCLUSIONS

De nombreuses méthodes en laboratoire de la toxicité des produits de combustion des matériaux ont été jusqu'à présent proposées. Les modèles feu sont variés, des plus simples comme les fours fixes (CORNISH,2) à de plus élaborés comme la chambre NBS (MORECI,3- EINHORN,4). OETTEL et HOFMAN(5) ont proposé le four mobile que nous avons utilisé. D'un autre côté, presque tous les essais font appel à des animaux,le plus fréquemment rats et souris, quelquefois des lapins. Les critères d'intoxication sont très variables. C'est souvent la DL50 (KIMMERLE,6 - CORNISH,2 - HERPOL,7). Des critères sublétaux sont également envisagés tels que la dépression ventilatoire (ALARIE,8), un réflexe d'évitement conditionné (PACKHAM,9), un temps d' incapacitation (CRANE,10) en cage à écureuil, les modifications de l'ECG (GAUME,11). Nous avons déjà proposé une méthodologie basée sur des déterminations physiologiques et biochimiques chez les animaux trachéotomisés (Physiogramme) (TRUHAUT,12 - JOUANY,13).

Le but de ce travail a été double. D'abord essayer, de façon simple, de prendre en compte un nombre assez

élevé de variables, en les hiérarchisant en fonction de leurs significations, puis en transformant les valeurs brutes en valeurs centrées réduites permettant des comparaisons inter-variables, puis leur association dans un index global conduisant à une possibilité de classement des matériaux. L'emploi de l'indice de PEARSON (χ^2) sur les valeurs centrées réduites augmente le pouvoir discriminant entre matériaux voisins. Mais il a aussi l'avantage de bien mettre en évidence des relations entre certaines variables privilégiées, autorisant ainsi certaines interprétations.

Ensuite, ces essais cherchent à élaborer une méthodologie relativement simple mais prenant en compte plusieurs paramètres fondamentaux indispensables. Ainsi, le four asservi apporte un élément de jugement important qui est la vitesse de combustion des matériaux. Les intoxications des animaux sont par ailleurs beaucoup plus régulières. La mesure d'un temps d'incapacitation via le siamographe proposé est facile à mettre en oeuvre et permet aussi la détermination des DL50. L'analyse des liquides de lavage pulmonaire donne de bonnes indications pronostiques et paraît devoir être systématiquement intégrée.

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However, the protection of the crew and passengers is not the only concern in dealing with the toxic hazards in aviation. Responsibilities include research to address the biomedical aspects of occupational health and safety standards, toxic substances, environmental impact criteria and classification of transportation.

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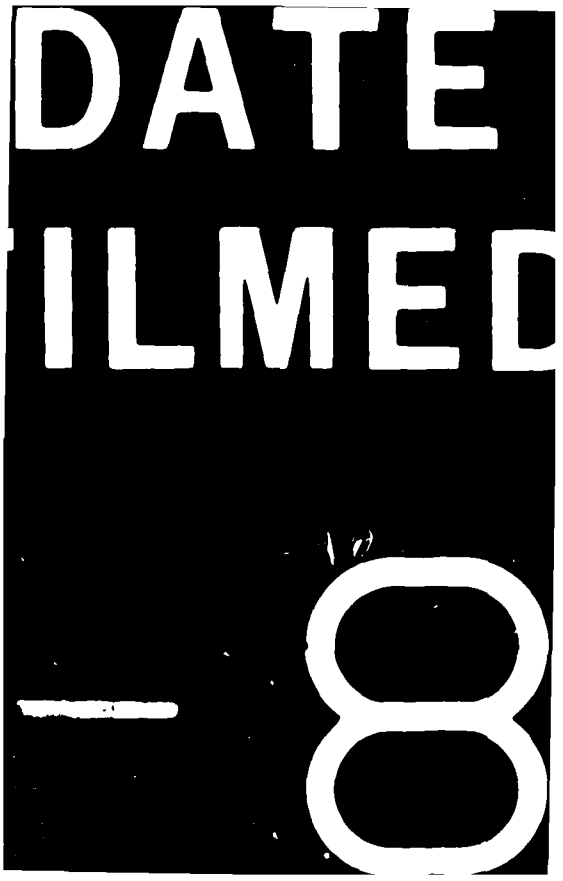
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